OBSERVATIONS ON THE EFFECTS OF REMOVAL OF SODIUM AND CHLORIDE FROM THE DIET

by

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CONTENTS

GENERAL INTRODUCTION

1. IMPORTANCE OF SALT

The literature of the ancients is full of references to the extreme importance of a salt supply. Aristotle relates how the Chaonims of Epirus had a salt spring which ran into a stream where there were no fish to be found. Legend had it that the Gods, in benevolent mood had allowed the people to have salt instead of fish and, indeed, the Gods many centuries ago were worshipped as givers of bread and salt.

In the light of modern knowledge it seems reasonable to theorise that this emphasis on the value of salt in ancient times stemmed from the prevailing climatic conditions. The ancient civilizations existed in hotter climates than we do. Their peoples, therefore, presumably perspired a great deal more and had large quantities of salt to replace in their bodies in an age when common salt was not so plentiful as it is to-day.

It is true that foods of animal origin generally contain enough salt to make its addition a luxury rather than a necessity. It is possible for man to live on most flesh foods without added salt. Milk certainly contains enough salt for the growing animal. It seems that, millions of

years ago when agriculture was introduced, our ancestors changed their dietary habits and developed a craving for salt which, to-day, is part of our twentieth century heritage.

The salt content of the diet of most mammals is low. Herbivores do not ingest more than 200 mg. salt per 100 calories and even less is obtained by birds and such mammals as the great apes which live on fruit, nuts and seeds. It is an enlightening fact that an American labourer getting a diet which provides him with 3000 calories a day probably takes in 16 to 24 g. salt which is as much per kilogram per day as the walrus (living on shellfish), 25 times more than the lion and 200 times more than his closest mammalian kin - the gorilla, orangutan and chimpanzee. (Dock 1950).

In modern times it is all too easy to forget the value of salt when the normal diet is never lacking in this commodity. In western civilizations, the average daily intake lies between 10 and 15 g. and the body can function normally on a diet containing much smaller amounts. (Duncan 1947).

2. IMPORTANCE OF SALT IN NUTRITION

There is an abundance of scientific literature to illustrate the fact that sodium chloride is an

essential substance in nutrition.

As early as 1818 Branche observed that salt deprivation resulted in extreme weakness, anaemia, albuminuria and oedema, but it seems certain that the cases which led Branche to his conclusions involved other serious dietary deficiencies and that salt deprivation played only a secondary role. (Chapman & Gibbons 1950).

In 1918 Osborne and Mendel concluded from their classical experiments on nutrition that failure of growth in the whole organism results when the salts of chlorine sodium, magnesium, potassium, calcium and phosphorous are deficient in the diet. They demonstrated that, for each of these elements, there is a minimum quantity which must be present in the diet for the normal development of growing animals.

More recently it has been shown that a diet containing only traces of sodium is ultimately fatal to animals. This was proved by Orent-Keiles et al in 1937 who further demonstrated that growth is not retarded to the same extent when both sodium and chloride are absent from the diet as when sodium deprivation alone is undergone. It is, of course, entirely wrong to think of sodium and chloride in the body as the single substance sodium chloride. The dietary supply of salt is also separated into its constituent elements each of

which has its own important role to play in body processes.

3. DISTRIBUTION OF SODIUM AND CHLORIDE IN BODY FLUIDS

The body of an average man of 70 kg wt contains approximately 50 litres of water. This is distributed between the intracellular and extracellular fluid compartments in the ratio of 2.5:1.

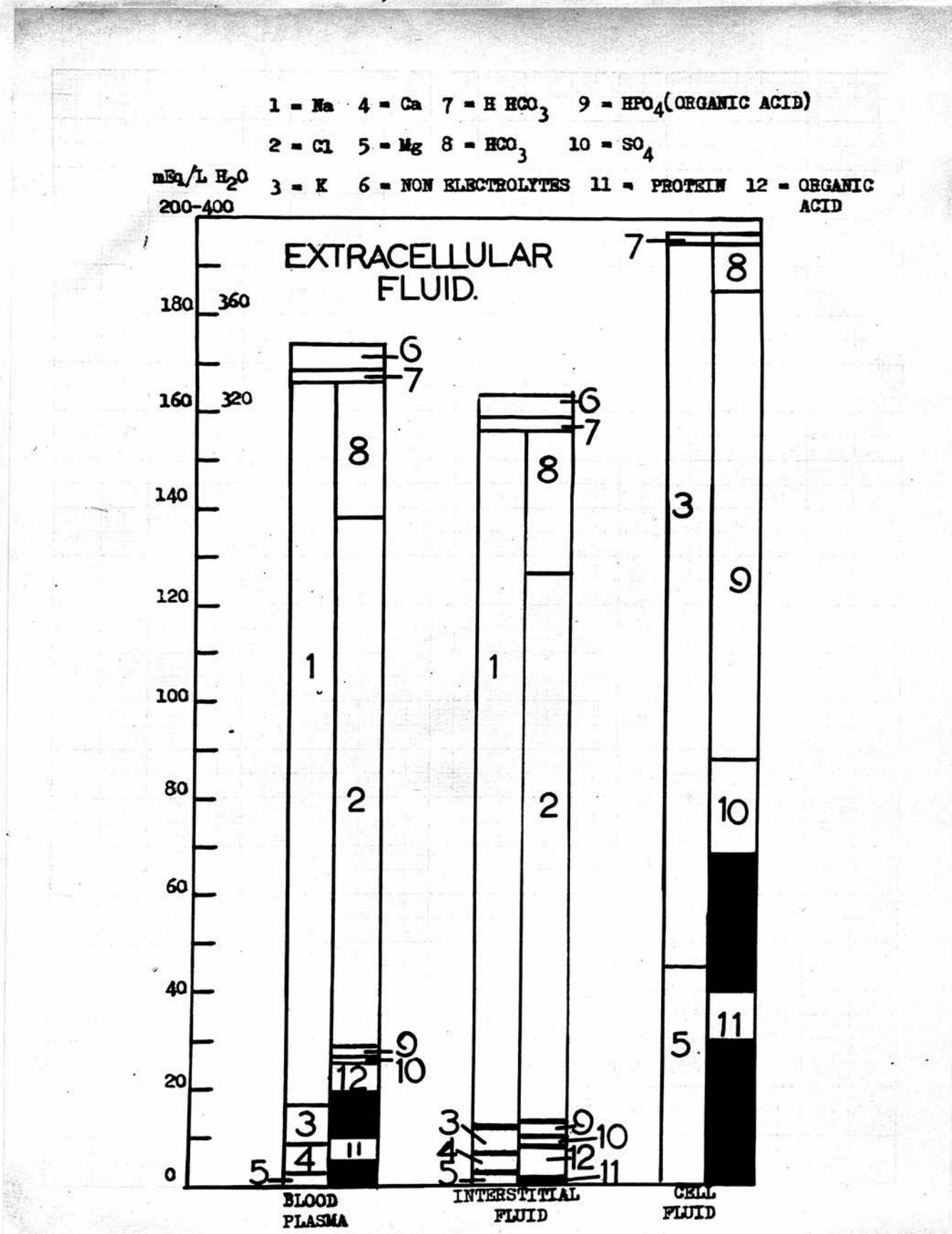
a. Extracellular Fluid Compartment

This includes the blood plasma within the blood vessels (approx. 3.5 L.) and the interstitial fluid lying between the tissue cells (10.5 L.).

Quantitatively sodium and chloride are the most important mineral constituents of the extracellular fluids of the body (Fig. I reproduced from Gamble 1947). The electrolyte composition of plasma and interstitial fluid is very similar. In each sodium is the principal cation and magnesium, calcium and potassium are present in smaller quantities. The main anion is chloride and bicarbonate takes second place. The concentration of both these ions is identical in plasma and interstitial fluid. Differences in composition are evident in the protein content

Figure 1

Comparison of electrolyte composition of blood plasma, interstitial fluid, and cell fluid. (Gamble, 1947).



of the two fluids. This is much greater in plasma which, therefore, contains extra cations to balance the extra protein anions.

Between plasma and interstitial fluids water and crystalloids can move freely and equilibrium is maintained between the two chiefly through the osmotic pressure of the plasma proteins which cannot pass freely to interstitial fluid.

b. Intracellular Fluid Compartment

The volume of fluid in the cells of the body amounts to about 36 litres in a normal person. In this compartment potassium is the dominant cation. Magnesium is present and very low concentrations of sodium. Snyder and Katzenbolgen (1942) established the sodium content of red blood corpuscles to be from 4 - 6 m. equiv. per litre. The principal anion is phosphate followed by protein, sulphate and bicarbonate in that order. The concentration of chloride ions is exceedingly low.

The cell membranes are freely permeable to water but only selectively so to electrolyte ions. Between the extracellular and intracellular fluid compartments a dynamic equilibrium therefore exists, controlled by the osmotic pressures of the

fluids within and without the cells. Any disturbance of osmotic balance between the fluids results in transfer of water from that of lower tonicity to that of higher tonicity.

4. REGULATION OF BODY SALT CONTENT

It is a matter of primary importance to the organism that the extracellular fluids should remain constant in composition since they form the external environment for the cells. Consequently the body content of sodium and chloride ions must be maintained within narrow limits.

Salt is supplied in the diet and excreted in (a) sensible sweat, (b) in faeces and (c) in urine. It is also temporarily lost from the body in the internal secretions.

The personal taste of the individual makes the amount of salt ingested daily a very variable quantity so that this factor is obviously unimportant in considering the means by which regulation of the sodium chloride content of the body is effected.

Neither may the loss of salt through the agency of sweat be considered as an important regulating mechanism since the quantity lost by this outlet is determined more by the need for maintaining body temperature within narrow limits than by the need to lose or conserve salt.

The amount lost in the faeces is so small as to be negligible.

Under normal conditions of gastro and intestinal function, the amounts of sodium and chloride excreted in the urine depend entirely on the amounts ingested in the preceding few days. When the dietary supply of salt is insufficient, and providing the water intake is maintained, the urinary levels of the two elements fall considerably and may reach negligible proportions if the restricted intake is severe and prolonged. Conversely the addition of a large quantity of salt to the diet of a normal individual will result in increased amounts of sodium and chloride in the urine until the excess has been excreted.

The responsibility for maintaining a definite constancy of sodium and chloride as well as of fluid in the body devolves, therefore, mainly upon the kidney. This renal action is, however, supplemented by the speed with which water and salt diffuse into the tissue spaces which act as buffers diminishing the disturbances due to sudden influx of water or salt into the plasma.

5. RENAL EXCRETION OF SODIUM AND CHLORIDE

Glomerular filtration is the physical process of ultrafiltration of plasma water and contained solutes through the glomerular membranes as the

blood passes through the glomeruli of the kidney. The glomerular filtration rate (G.F.R.) has been determined by means of inulin, creatinine, mannitol, thiosulphate or allantoin and the normal values vary between 90 and 150 ml per minute. As this large volume of fluid passes through the kidney tubular urine is formed which has a minute volume of less than 5 ml and which is very different from glomerular filtrate in composition. If the G.F.R.= 100 ml/min and the serum Na concentration = 140 mEq. per litre, the amount of sodium filtered per minute is 14 mEq. approximately (neglecting the error from plasma proteins and from Donnan effect on Na in the two phases). Na excretion, however, varies between 0.003 and 0.3 mEq. per minute. It follows that most of the filtered sodium is reabsorbed.

It is suggested by Wesson et al (1948) and Wesson and Anslow (1952) that sodium, together with chloride and bicarbonate is actively reabsorbed in the proximal tubule, with the formation of a slightly hypotonic fluid. On passage through the thin limb water diffuses back into the plasma and an isotonic urine is delivered to the distal tubule where sodium and water are actively but independently reabsorbed. Reabsorption in the thin limb accounts for approximately $\frac{7}{8}$ of the filtered load. The remaining $\frac{1}{8}$ is presented to the distal tubule which has only a limited capacity for the reabsorption of both sodium and water, the limiting

factors being the maximal rates of tubular transport. Normally Na is here reabsorbed to the extent of $\frac{1}{8}$ of an average Na load so that at low loads the reabsorption of the ion may be complete but at high loads the excess will escape reabsorption and find its way into the urine. Distal reabsorption of water is activated by the antidiuretic hormone of the posterior pituitary which, with the osmoreceptors of the supraoptico-hypophyseal system comprises the mechanism for the control of water equilibrium.

This theory of the renal excretion of sodium is based on evidence gained from micropuncture studies in mammals (Walker et al 1941) and from observations on osmotic diuresis (Wesson et al 1948, Wesson and Anslow 1948). Black (1952), however, believes that another factor should be included - the variability in tubular function for reabsorption of sodium. There is a diurnal variation in sodium excretion which cannot be accounted for by the very small changes in G.F.R. during sleep (Black 1952, Stanbury and Thomson 1951). Moreover normal individuals who are made to hyperventilate have an increased urinary excretion of sodium without any increase in the filtered load of sodium (Sirota et al 1950). The postulation of reabsorption of a constant amount by the distal tubule and a constant fraction by the proximal tubule has also been

criticised by Selkurt (1954) who favours the view that the amount of sodium excreted is a changing fraction of the amount filtered.

Plasma sodium is largely linked with chloride and bicarbonate. The sodium ion in the reabsorbate and in the urine will, therefore, be predominantly associated with these two anions and excretion of chloride will, in general, closely follow that of sodium. This is not, however, always the case. Chloride may also be excreted with K and NH_3 (Smith 1951). Excretion of chloride is dissociated from that of sodium after ingestion of bicarbonate and it has also been shown that addition of extra salt (10 g.) to a normal diet may result in retention of sodium without chloride or vice versa (Goldzieher & Stone 1949).

6. INFLUENCE OF ASSOCIATED ANIONS ON RENAL EXCRETION OF SODIUM AND CHLORIDE

Associated anions influence sodium excretion by the kidney mainly due to quantitative differences in their reabsorptive systems. Thus $NaHCO_3$ is reabsorbed at the rate of 2.6 to 2.8 mEq./100 ml. of filtrate per minute and chloride at a rate of 10.5 mEq./100 ml. of filtrate per minute (Pitts et al 1949). It follows that the quantity of sodium lost from the body will be greater if a certain amount is infused as $NaHCO_3$ than if the same amount is administered as NaCl, since the greater number of

HCO_3 ions which must be excreted require a proportionately greater number of cations for linkage.

With anions which are only slightly reabsorbed an even greater loss of sodium would be expected. This has, in fact, been demonstrated experimentally (West et al 1952).

7. HORMONAL INFLUENCE ON RENAL EXCRETION OF SODIUM AND CHLORIDE

The active principals of the adrenal cortex exert an important effect on renal tubular activity in reabsorbing sodium and chloride.

In adrenal insufficiency failure to retain sodium and chloride is evident. Excessive quantities are lost in the urine and a decrease in plasma levels may result.

The precise nature of the defect is still a matter for conjecture but it appears to be due to a defect in renal tubular function. It has been suggested that the excessive urinary loss of salt evident in adrenalectomised animals and in patients suffering from Addison's disease is not a direct result of lack of adrenocortical hormone but is due, in part at least, to disturbance of the hormonal balance which normally exists between the anti-diuretic principle of the pituitary gland and the salt retaining principle of the adrenal cortex.

8. SALT AND HYPERTENSION

Hypertension is one of the most common diseases

affecting the peoples of western civilizations. It is, however, apparently rare and levels of blood pressure are generally lower in the natives of the Orient and in the undernourished populations of the tropics (Dock 1950).

Racial characteristics may, of course, play a part in producing this difference. Genetic factors involving endocrine and nervous response to stress or simply widely divergent philosophies of life could provide at least part of the explanation. The inscrutability of Oriental peoples, their calm response to life's exigencies and their fatalistic acceptance of happiness and misfortune are almost legendary. These attributes must render their existence quieter and less disturbed than that of inhabitants of western civilizations who do not possess the same defence against adversity and stress.

In order to prove or disprove the influence of racial make-up on incidence of hypertension, reliable and extensive data would be necessary on the blood pressures of Orientals and tropical natives living in western populations together with similar figures for Europeans transplanted to the countries of the East. It would, of course, be a necessary condition that each group of migrants should live the life of the natives of the new region. Such figures are not available but it has been fairly

well established that negroes in Africa display much lower blood pressure levels than members of the same race who have migrated westwards. Conversely Europeans living in the tropics appear to demonstrate no increased resistance to the development of hypertension. (Dock 1950)

These latter facts would be explained if the differences in blood pressure levels were due to dietary factors. The negro living in the west consumes the food of the milieu in which he finds himself, but the European living in the tropics does not, as a rule, share the diet of the native tribes. Most of these people live on an exceedingly low intake of both protein and salt since they exist largely on cassava and sago from which sodium and protein are removed in the course of their preparation as food. (Dock 1950)

If diet is, in fact, responsible for the low incidence of hypertension in some parts of the world, it still remains a matter for conjecture whether the effective agent is low sodium intake, low protein intake or a combination of both.

There is considerable experimental evidence to indicate that hypertensive conditions may be sensitive to dietary salt and the role of sodium in causing the disease has attracted the attention of research workers since the beginning of the present century. This interest has been reflected in the

various applications of restriction of salt intake as a therapy in treating hypertension and allied conditions.

9. DIETARY SALT RESTRICTION AS A THERAPY

Although salt deprivation was not applied to the treatment of hypertension specifically until 1904, the belief that restriction of the salt intake is beneficial to patients suffering from various types of cardiovascular renal disease dates from the mid-nineteenth century. Indeed as early as 1831 Chrestian virtually proposed the therapy when he advocated a milk diet for all diseases associated with oedema, and during the second half of the century research workers were active in examining the role of salt in such conditions.

In 1850 Redtenbacher observed that febrile disease is often associated with low chloride excretion and in 1871 Salkowski proved the simultaneous retention of the sodium ion. In 1898 von Koranji found chloride retention in cases of cardiac failure and in the following year Carrion and Hallion demonstrated that both pulmonary and peripheral oedema could be produced in dogs by the intravenous injection of salt solution. In 1901 Achard and Van der Bergh independently confirmed the retention of chloride in febrile disease, cardiac failure and nephritis and in 1903 Widal and Lemierre found that addition of salt to the diet of dogs

could result in the formation of oedema and limitation of the intake was followed by disappearance of the accumulated fluid. (Chapman & Gibbons 1950).

The Frenchmen Ambard and Beaujard (1904) were the first to treat hypertensive patients by means of dietary salt restriction, utilizing a diet providing about 3 g. per day and comparing it with a control diet which contained 14 g. These workers observed a tendency of the blood pressure to go down during periods of salt restriction but they believed that the chloride ion was responsible for the favourable effects. The pressure of the blood was conceived to be very sensitive to the passage of chloride into the organism and to whether it was eliminated or retained. Permanent hypertension was thought to be an expression of non-adaptation to saturation of the organism with saline.

During the years between 1909 and 1920 the observations of Blum and his co-workers indicated that the emphasis on the chloride ion as the beneficial agent in cases of renal vascular diseases treated with low salt therapy was misplaced. In 1909 Blum noted that diabetics on a low caloric and high sodium bicarbonate intake often developed oedema. He reached the conclusion that by feeding large quantities of a sodium salt it is possible to induce water retention to the point of oedema and in

1921 he showed that both sodium chloride and sodium bicarbonate could produce this effect. Finally in 1921 Blum et al made the suggestion that the sodium ion was the important factor. (Chapman & Gibbons 1950).

In spite of these findings the opinion of the early French workers that the chloride ion was the one on which attention should be focussed was still the belief of Allen and Sherrill who, in 1922, succeeded in lowering the blood pressure of hypertensive patients with a diet low in salt. Despite their favourable findings the work of Allen and Sherrillwas inconclusive because it was uncontrolled. During the next few years this work was repeated by other investigators some of whom claimed to confirm the earlier findings but the criticism of lack of control can be directed at all these experiments.

Gradually the form of therapy fell into disuse and interest in the subject flagged.

10. THE RICE DIET

In 1944 Kempner's papers began to appear in the Medical Press in the United States and, once more, the controversial question of salt restriction in relation to hypertension was forced into a position of prominence in medical circles.

Kempner's rice diet was not originally introduced as a means of restricting salt intake in

particular. The essential features were, however drastically, reduced contents of salt and protein and minimal amounts of all other nutrients.

He observed that the rate of deamination of amino acids by kidney tissue was related directly to the prevailing oxygen tension and he reasoned that if some pathological condition had caused a decrease in oxygen supply to any one kidney cell, it might still be possible to increase the concentration of oxygen by decreasing the amount of work required from that cell. It follows, on this line of reasoning that reduction in the protein intake will decrease both amount of deamination required and amount of oxygen consumed by renal tissue. Kempner arrived at similar conclusions from observations on the role played in renal metabolism by sugar, sodium bicarbonate and other normal constituents of the diet and proposed the rice diet as a means of reducing all factors of renal work to a minimum by cutting down food intake.

The Kempner regime was rigid. Patients received a diet which provided them with 2000 calories and contained 5 g. fat, 20 g. protein derived from rice and fruit and not more than 0.2 g. chloride and 0.15 g. sodium.

In a paper published in 1946 Kempner claimed that in 203 out of 322 patients having renal metabolic disfunction the rice diet led to objective

improvement when other forms of therapy failed. Of 100 patients with primary kidney disease, 65% showed improvement on the rice diet. Of 22 patients where a diagnosis of hypertensive vascular disease was made 62% improved.

The author also reported improvement in the eyegrounds, reversal of abnormal electro-cardiograph patterns and diminution in the size of the heart in many of his patients.

From the biochemical aspect he observed that, in patients on a rice fruit diet, sodium and chloride excretion became minimal and that the concentration of K was higher in the urine than in that of a person on a normal diet. This latter finding might be expected on a high fruit intake.

Most of Kempner's claims in his early papers were questioned by later workers, many being of the opinion that any depressor effects of the rice-fruit regime might well be due to the rest, quiet and autosuggestion of being treated which a stay in hospital offers. Criticism has also been levelled at the diet on the grounds that the protein and fat restriction are too rigid, at least 45 g. protein being required to maintain equilibrium with the proteins of the body.

It appears, too, that Kempner's original reasoning lacked experimental support. There seems to be little evidence to show that renal metabolism

is significantly raised by increasing the salt and fat contents of the diet and experimental results have indicated that when osmotic work done in excreting urea is increased fifty times, renal oxygen consumption increases less than 25%. (Dock 1933). Further no demonstrable increase in renal oxygen consumption is evident when rats are changed from a protein free to a 74% protein diet, although since renal hypertrophy occurs some change in renal metabolism must take place. (Dock 1931).

Despite adverse criticism, however, there seems to be little doubt that Kempner's rice-fruit diet can effect improvement in some hypertensive conditions. Chapman (1950) reported a lowering in blood pressure of hypertensive patients treated with this therapy and he refuted the argument that a hospital regime might be the ruling factor when he pointed out that there was no decrease in blood pressure, after the first days, until the rice diet started and, though day to day variations were sometimes large they were never systematic.

11. THE EFFECTIVE AGENT IN THE RICE DIET

In 1945 Grollman and his associates observed that drastic reduction in sodium intake resulted in a fall in blood pressure in some hypertensive patients and they believed it probable that the beneficial effects of Kempner's rice diet were due to the restriction of salt. This belief was supported by

the work of Perera and Blood (1947) who demonstrated the sensitivity of hypertension to dietary salt when they found that rigid restriction of NaCl intake could lower the blood pressure of patients with hypertensive vascular disease and conversely that under similar conditions large amounts of dietary sodium chloride could exert a pressor effect in hypertensive patients.

In 1950 Chapman showed that the addition of 40 g. salt free protein daily to the rice diet did not affect the improvement obtained in treating cases of hypertension. This result, combined with the earlier findings seemed to establish sodium restriction as the operative factor and further proof was added in 1951 by Dole et al. The latter workers found that restriction of dietary sodium to 7 mEq./day caused statistically significant reductions in the average blood pressures of hypertensive patients and emphasized that this result was indeed due to limitation of sodium in the diet since no changes in blood pressure were observed over a period of 2 - 4 months during which the low Na diet was supplemented with salt tablets.

There is little doubt, therefore, that restriction of dietary salt may be an effective therapy in cases of hypertension but much further experimental work is necessary before a satisfactory explanation can be postulated for its

beneficial effects. It is generally agreed, however, that such treatment is ineffective if the disease occurs without renal involvement.

12. DISTURBANCES IN SALT METABOLISM IN HYPERTENSION

In essential hypertension a lowered rate of glomerular filtration has been reported. Normal sodium balance is a general finding (Selkurt 1954). It follows that changes must take place in the processes involved in regulation of the body content of the ion. In the literature variations from the normal in renal treatment of both sodium and chloride have been reported.

Farnsworth and Barker (1943 A & B) suggested that tubular reabsorption of chloride is depressed in hypertension because they found that the ratio of the concentration of chloride in urine to the concentration in plasma was higher than in normal for any given urine to plasma concentrations of inulin.

Perera and Blood (1946) have reported that the changes which take place in tubular reabsorption of chloride in human hypertension differ from those that are shown in normal subjects when dietary sodium is abruptly restricted.

It has also been demonstrated that the hypertensive shows an excretion of sodium and chloride which is higher than normal in water and mannitol diureses and the suggestion has been made that this

effect is one of the earliest signs of renal tubular damage in hypertension (Weston et al 1950).

The possibility exists that in hypertension there is some impairment of the mechanism whereby the adrenal cortex influences regulation of excretion of Na by the kidney. This has been the subject of work by Perera and Blood (1947) and Schroeder (1948) and will be considered more fully later.

13. THE PROBLEM

The problem of sodium metabolism in health and disease forms to-day the subject of much discussion and conjecture, and investigation of the diverse and complex factors involved constitutes a vast and active field of research work. During the past twenty years knowledge of the subject has greatly increased and theories have been formed, discarded and reformed to fit the established facts. Many questions, however, remain unanswered and a great deal of work is still to be done before the picture of sodium metabolism is complete.

The experimental work which is reported here was undertaken in an attempt to gain further information on certain aspects of the problem. It was concerned with salt conservation in normal and hypertensive individuals on a diet restricted in sodium chloride and included observations on the effects of this regime on the concentration of plasma electrolytes and urinary excretion of electrolytes and

corticosteroids.

The work is presented in two sections. In section 1, the observed electrolyte changes are considered and discussed, while section 2 is devoted to examination of the role of the adrenal cortex in producing these electrolyte changes.

SECTION 1 - ELECTROLYTES

1. INTRODUCTION

The present study was concerned with the response of hypertensive and normal subjects to the sudden withdrawal of salt from the diet. It was hoped that information might be gained concerning the mechanism by which the kidney achieves conservation of body salt and maintenance of normal sodium balance under such conditions. Further, it was considered that any variation in response to this stimulus between the hypertensive and normal persons might provide an indication of abnormal salt metabolism in the hypertensive condition.

2. PROCEDURE

The experimental subjects formed two groups. The members of the first group were six patients suffering from uncomplicated essential hypertension and the second group comprised four patients who had recovered from minor complaints prior to the period of study and two healthy volunteers, all showing normal blood pressure readings.

The age range was similar in the two groups, each of which included three male and three female subjects of normal weight with regard to height. All these persons were free from albuminuria and showed normal levels of blood urea nitrogen. In no

TABLE I

Relevant Clinical Data

Hypertensive Subjects	Age	Sex	Blood Pressure. Average range during 7-10 days control period before salt withdrawn from diet.	
			Systolic	Diastolic
1	49	F	210±10	128±13
2	43	F	205±15	110±5
3	40	M	152±8	110±7
4	43	M	169±10	103±8
5	40	F	160±10	108±5
6	46	M	167±13	105±10
Normal Subjects				
1	35	F	115±0	68±2
2	43	F	137±8	87±5
3	54	M	110±5	65±5
4	50	M	118±5	67±5
5	47	F	130±5	80±5
6	34	M	120±5	75±5

case was there evidence of cardiac or endocrine disease and renal function, as shown by urine concentration tests and by examination of the clearance rates of inulin or creatinine was, without exception, normal. The essential clinical details are summarised in Table 1.

For the duration of the experiment each subject lived as a patient under the conditions of a hospital regime and was, therefore, largely at rest and free from responsibility. On admission to hospital the patients were fed a rice-fruit diet containing constant amounts of all nutrients with added sodium chloride in the form of rice paper cachets of salt until excretion of the electrolytes under study in the urine had become stabilised. The cachets of sodium chloride were then omitted from the diet and the patients continued on a salt free food intake, which was rigid and strictly supervised, until the termination of the experiment.

Twenty four hour collections of urine were made daily and all estimations were carried out as soon as possible after each specimen was complete. Blood was withdrawn from the patient in the fasting condition before breakfast and all analyses were carried out on serum.

3. DIET

The diet was based on the rice-fruit diet of Kempner, and was completely constant throughout the

period of study for each experimental subject.

In order to ensure constancy in potassium intake it was necessary to include only one type of fruit in the diet of each person and to utilise only one batch of tinned fruit juice in each case. The variation in the potassium content of the fruit juice from different batches was responsible for the variation which was evident from patient to patient in the amount of this ion ingested. In three cases (Hypertensive case no. 6, Normotensive case nos. 4 & 6) where the quantity of potassium obtained from the food was small, this was supplement-ed by the addition of fixed amounts of potassium citrate to the fruit juice.

The total number of Calories provided by the daily food intake varied from 1500 to 2500 according to the weight and size of the subject under study. In every case the Caloric value of the diet was sufficient to maintain weight and all subjects were in nitrogen balance. Added protein was given in the form of Casilan (Glaxo Laboratories) in amounts varying between thirty and fifty grammes.

After removal of the sodium chloride cachets from the food the total intake of sodium in twenty four hours was calculated to be 10 mEq. and the total chloride intake 20 mEq. Analysis of a complete twenty four hours diet gave the following results:

sodium - 13 mEq.

chloride - 18 mEq.

TABLE 2

Salt Free Diet. Typical Menu for 24 hrs.

	Sugar	Jam	Marmal-ade	Salt free Bread	Salt free Butter	Rice	Milk	Eggs	Fruit	Fruit Juice
Breakfast	16 g.		15 g.	30 g.	8 g.		50 g.	1	100 g.	
10.30 a.m.	15 g.									150 g.
Dinner	25 g.					50 g.			100 g.	150 g.
Tea	10 g.	15 g.		30 g.	8 g.		50 g.			
Supper	40 g.					50 g.			100 g.	150 g.
9.00 p.m.	15 g.									150 g.

Carbohydrate	364 g.	(approx.)	= 1600 Calories
Fat	29 g.	(approx.)	= 300 Calories
Protein	31 g.	(approx.)	= 100 Calories
Total Caloric Intake		(approx.)	= 2000 Calories

In Table 2 are detailed the meals received by one of the hypertensive patients (case no.6) during the course of a day after salt intake had been stopped.

4. METHODS

Sodium: Two methods were utilised for the estimation of sodium.

1. Gravimetric Method (Butler & Tuthill 1931).
2. Barclay Flame Photometer. Internal standard method utilising lithium as the internal standard.

In the early cases (Hypertensive cases 1, 3 & 4, Normal case 2) sodium was estimated by method 1 and in the later cases by method 2.

Potassium: As for sodium, two methods were employed, method 1 being utilised in the early cases.

1. Titrometric method (Shol and Bennet 1928).
2. Barclay Flame Photometer. Internal standard method utilising lithium as the internal standard.

Chloride: Titrometric method (Van Slyke 1923).

Calcium: Titrometric method (Kramer and Tisdall 1922, 1923).

Magnesium: Colorimetric method of Briggs (1922).

Inorganic Phosphate: Colorimetric method of Fiske and Subba Row (1925).

Inorganic Sulphur: Gravimetric method of Benedict

(1909).

Nitrogen: Microkjeldahl.

Inulin: Colorimetrically utilising the colour reaction with resorcinol and ferric chloride (Cole unpublished).

Creatinine: Determined using the NC - bacteria and the Jaffe reaction according to the method of Miller and Dubos (1937). (Modification of Lloyds reagent method of Haugen and Blegen 1953).

5. RESULTS

a. Weight Changes after dietary restriction of Salt.

All the subjects who were used in this study suffered a loss in body weight after salt was removed from the diet. The weight changes which occurred during the first 48 hr. after ingestion of salt had stopped are summarised in Table 3.

The average amount of weight lost by the members of the normotensive group was slightly greater than that lost, on an average basis, by the hypertensive subjects after 24 hr. of salt deprivation. The significance of this finding was evaluated from the formula $t = \frac{m_2 - m_1}{\sqrt{SE_2 + SE_1}}$ where m = mean, SE = standard error and t was checked against Fisher's tables. The difference between the two means was found to be statistically insignificant and remained so

TABLE 3

Weight Changes

Normotensive Subjects				Hypertensive Subjects		
Wt. prior to salt restriction Kg.	Wt. loss 24 hrs. after salt restriction Kg.	Wt. loss 48 hrs. after salt restriction Kg.	Case No.	Wt. prior to salt restriction Kg.	Wt. loss 24 hrs. after salt restriction Kg.	Wt. loss 48 hrs. after salt restriction Kg.
41.15	0.15	0.70	1	58.40	0.10	0.40
67.25	0.50	0.80	2	74.35	0	0.20
73.80	0.25	0.55	3	52.40	0.15	0.85
76.65	0.10	0.70	4	48.30	0.10	0.35
80.85	0.20	0.70	5	82.05	0.35	1.00
71.95	0.60	0.10	6	60.0	0.25	0.30
Average	0.30	0.59			0.16	0.52

	Mean Values for normotensive subjects Kg.	Mean Values for hypertensive subjects Kg.	Probability of difference between hypertensives and normals being due to chance
Wt. loss 24hrs. after salt restriction	0.30±0.2	0.16±0.15	0.2
Wt. loss 48 hrs. after salt restriction	0.59±0.25	0.52±0.32	0.7

To be significant P (Probability) must be less than 0.05

when sodium chloride had been lacking in the food for 48 hr.

b. Changes in urine volume after dietary restriction of salt.

Most of the experimental subjects showed a slight increase in urine volume during the first twenty four hours after dietary salt restriction and in some cases this increase was still evident after ingestion of salt had been stopped for forty eight hours. The changes are shown in Table 4. They are variable from case to case but average figures for the first twenty four hours are similar for the hypertensive and normotensive subjects. After forty eight hours the average figure for the normal cases is higher than such a value for the hypertensive patients. This difference was found, however, to be statistically insignificant when evaluated according to the formula stated above.

c. Urinary sodium excretion after dietary restriction of salt

After withdrawal of salt from the diet of each experimental subject the urinary excretion of sodium gradually decreased until it amounted to less than 10 mEq. per day. At this point equilibrium was practically restored between the amounts of sodium ingested and excreted, continued small losses in the urine, faeces and

TABLE 4

Changes in Urine Volume

Normotensive Subjects			Hypertensive Subjects		
Case No.	Change 24 hr. after dietary salt restriction ml.	Change 48 hr. after dietary salt restriction ml.	Case No.	Change 24 hr. after dietary salt restriction ml.	Change 48 hr. after dietary salt restriction ml.
1	+310	+70	1	+210	+130
2	-60	0	2	+200	+200
3	+100	+100	3	-160	-40
4	+110	0	4	+280	+280
5	+170	-300	5	+300	+120
6	+820	+1580	6	+530	+440
	+241	+241		+227	+190

	Mean Value for normotensive subjects	Mean Value for hypertensive subjects	Probability of difference between hypertensive and normals being due to chance
Change 24 hrs. after dietary salt restriction	241±210	227±93	0.9
Change 48 hrs. after dietary salt restriction	241±662	190±137	0.9

To be significant P must be less than 0.05

sweat causing only a slight negative balance with regard to this ion. This effect is shown for the normotensive cases in Fig. 2 and for the hypertensive cases in Fig. 3.

The response of the hypertensive patients to abrupt restriction of dietary salt is compared with that of the normal persons in Table 5. The urinary excretion of sodium prior to salt withdrawal is listed for all the subjects and three points are considered:

1. The amount of sodium remaining in the urine twenty four hours after salt withdrawal (expressed as a percentage of the mean daily excretion during the control period).
2. The total amount of the sodium loss during the period of negative balance which occurred after salt ingestion had stopped and before urinary sodium excretion had reached a minimum.
3. The number of days required to re-establish balance when salt was no longer present in the food.

It is evident that the mean urinary excretion of sodium prior to dietary salt restriction was similar in both groups. Average figures indicate that the amount of sodium remaining in the urine of the normotensive subjects and the total sodium loss

Figure 2

Normal Subjects. Urinary excretion of sodium during a control period and after removal of salt from the diet.

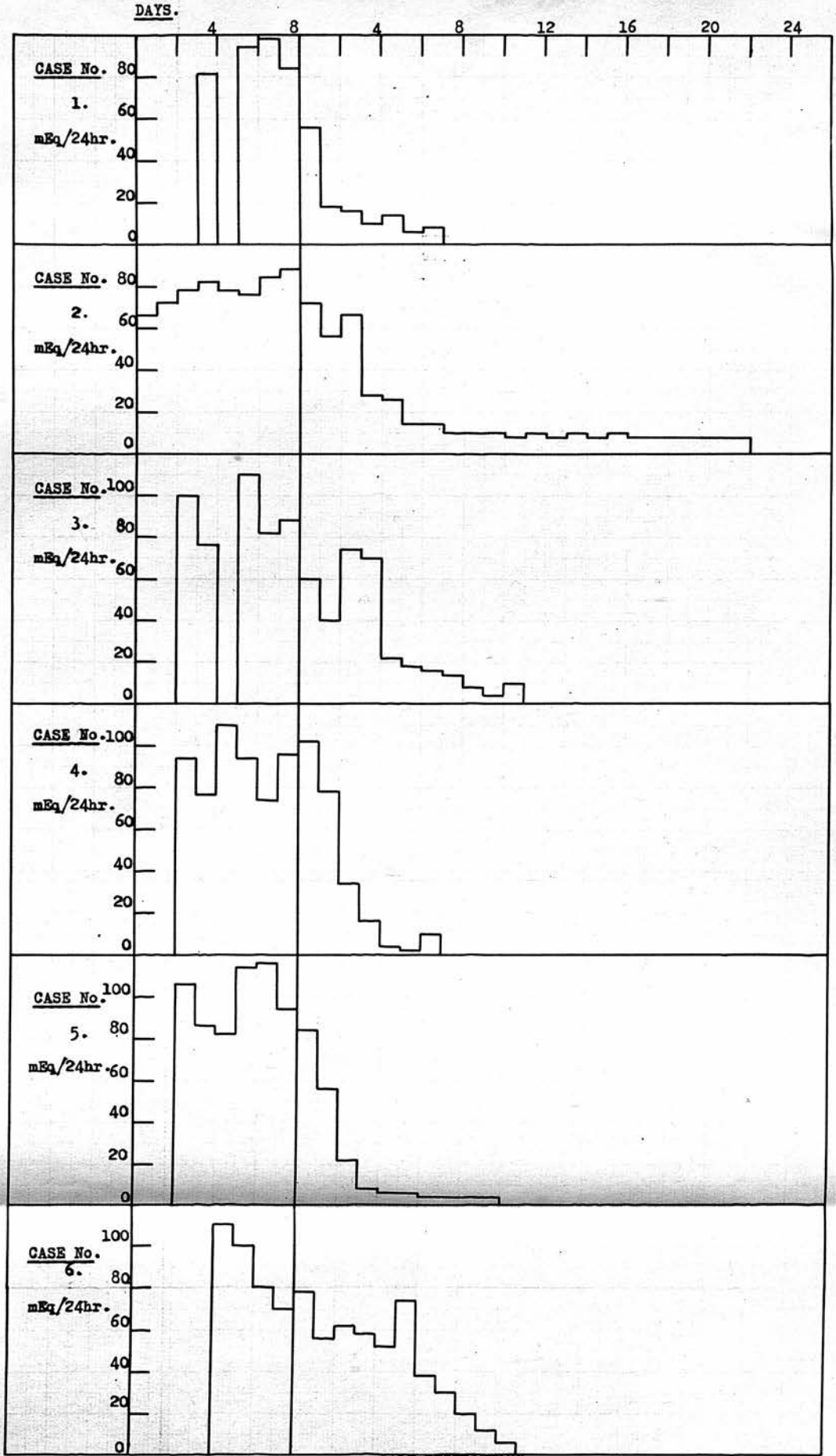


Table 5

Urinary Excretion Na

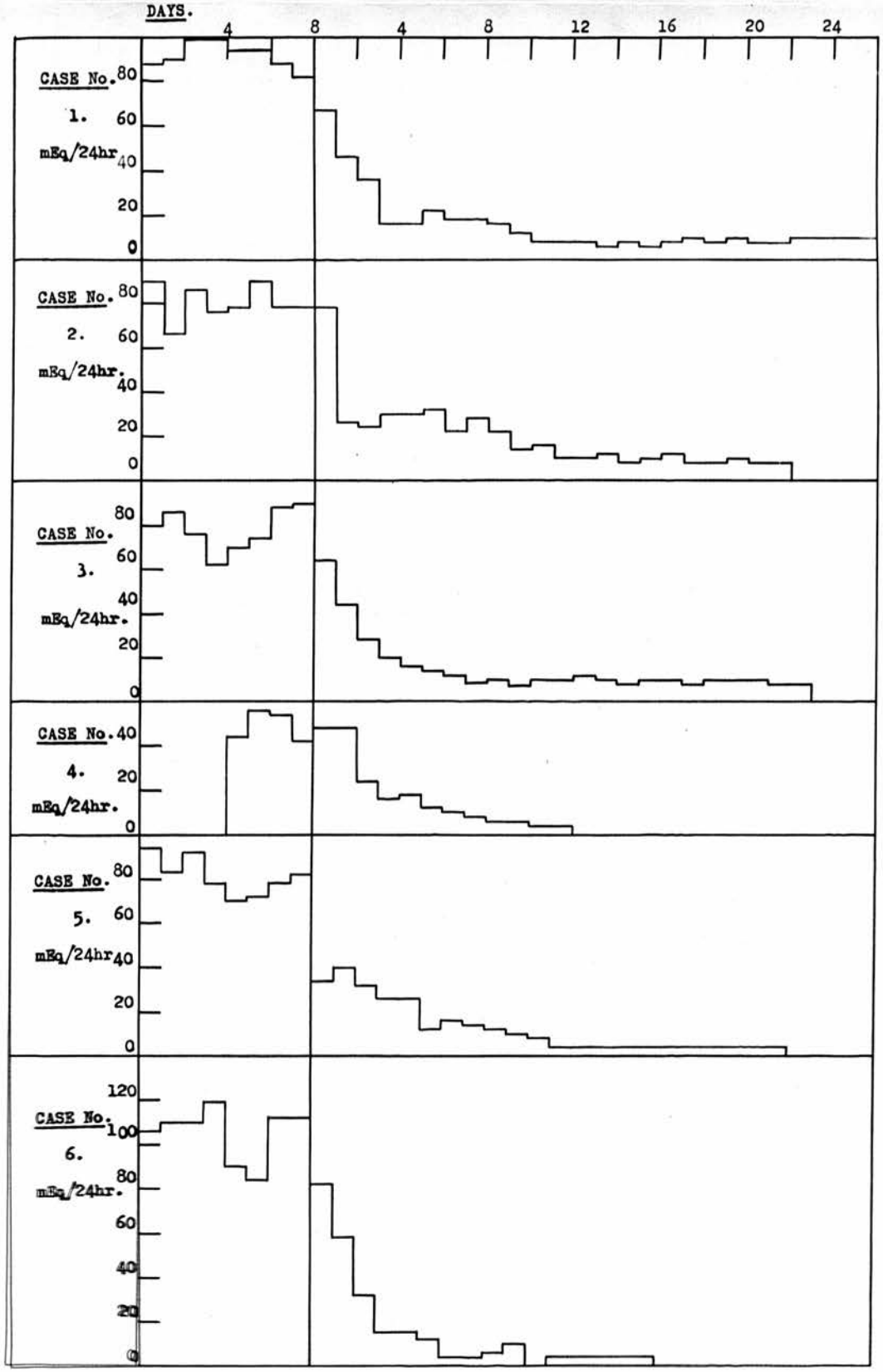
Hypertensive Subjects	Mean 24 hr. urinary excretion Na prior to salt withdrawal mg/24 hr.	Na remaining in urine in 1st 24 hr. after salt withdrawal. (% of mean before salt withdrawal)	Total Cumulative -ve Na balance after salt withdrawal M.eq.	No. of days taken to re-establish sodium balance aft salt withdrawal.
1	95.0	70.5	162	10
2	78.7	97.8	202	12
3	78.2	81.8	142	8
4	48.8	96.3	104	6
5	80.7	43.4	178	10
6	103.6	79.2	164	7
Average	80.8	78.0	159	9
Normotensive Subjects				
1	88.8	63.5	61	4
2	79.9	90.1	188	8
3	81.8	74.3	236	9
4	87.5	118.4	215	5
5	99.9	83.2	130	4
6	89.7	86.5	396	10
Average	87.9	86.0	204	7

	Mean value for Normotensive subjects.	Mean value for Hypertensive subjects	Probability of difference being due to chance.
Na remaining in urine in 1st 24 hr. after salt withdrawal (% of mean before salt withdrawal).	86.0±17	78.1±15	0.5
Total Cumulative -ve Na balance after salt withdrawal (M.eq.)	204±82	159. ±33	0.3
No. of days to re-establish balance after salt withdrawal.	7±2	9.±2	0.2

In order to be significant P must be less than 0.05

Figure 3

Hypertensive Subjects. Urinary excretion of sodium during a control period and after removal of salt from the diet.



demonstrated by these people were higher than were observed in the cases of hypertension which were studied. Further the period of time during which negative balance existed was shorter on a mean basis for the normal individuals than for the hypertensive patients. These differences, however, proved to be insignificant when evaluated according to the formula which has already been quoted in reporting the changes in body weight and urine volume. It is possible, of course, that a significant difference between the two groups lies in the fact that the normotensive subjects accumulated a greater mean sodium deficit than the hypertensive patients in a shorter mean time. This might suggest a slight quantitative difference in the response of normo- and hypertensive subjects to the conditions described.

d. Urinary chloride excretion after dietary restriction of salt.

Urinary excretion of chloride followed the same pattern as sodium after salt was removed from the food. Figures 4 and 5 show the decreased amounts excreted in the urine of the normotensive and hypertensive subjects respectively until balance was re-established between dietary intake and urinary output.

Table 6 shows the amount of chloride

Figure 4

Normal Subjects. Urinary excretion of chloride during a control period and after removal of salt from the diet.

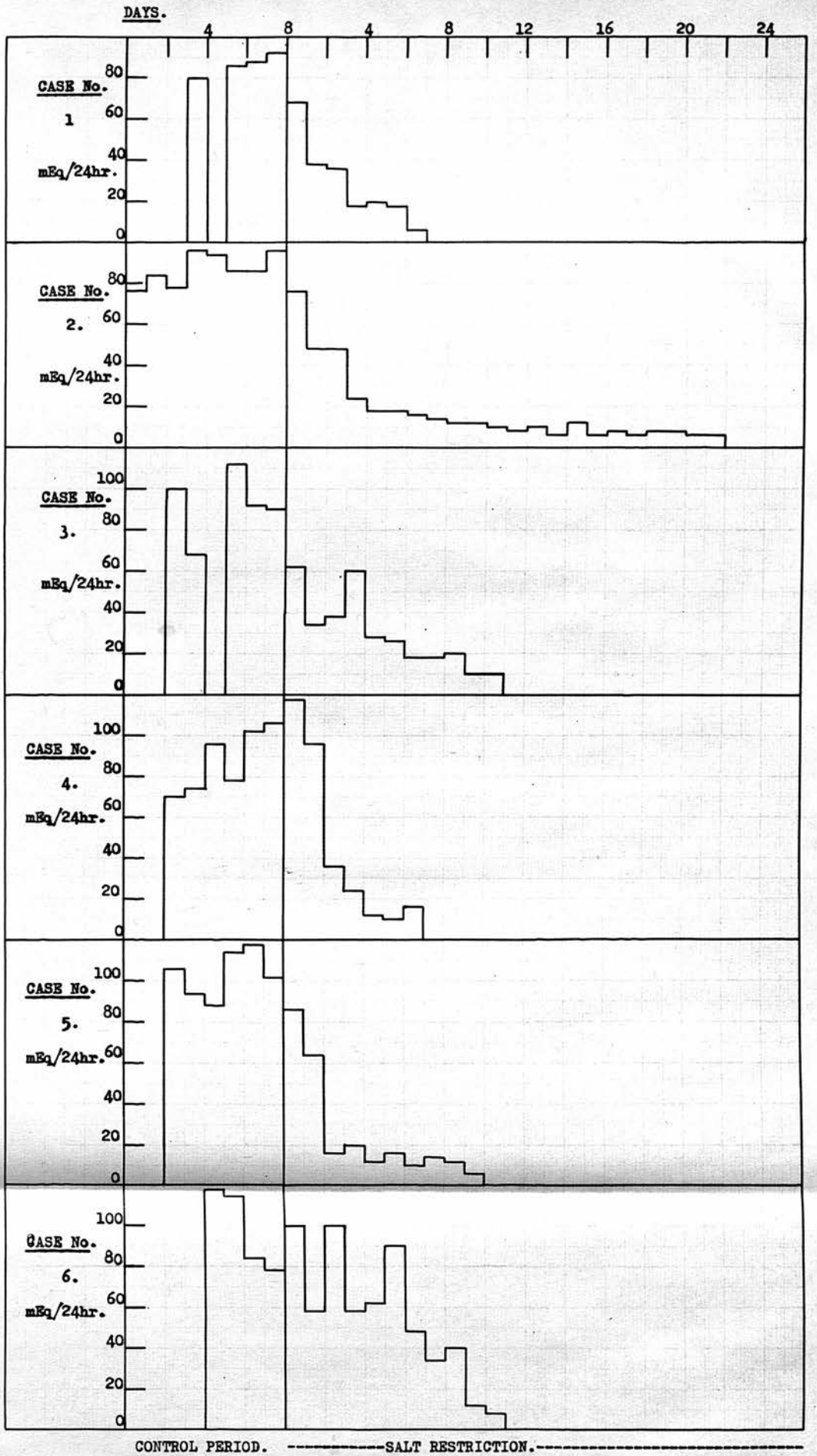


Figure 5

Hypertensive Subjects. Urinary excretion of chloride during a control period and after removal of salt from the diet.

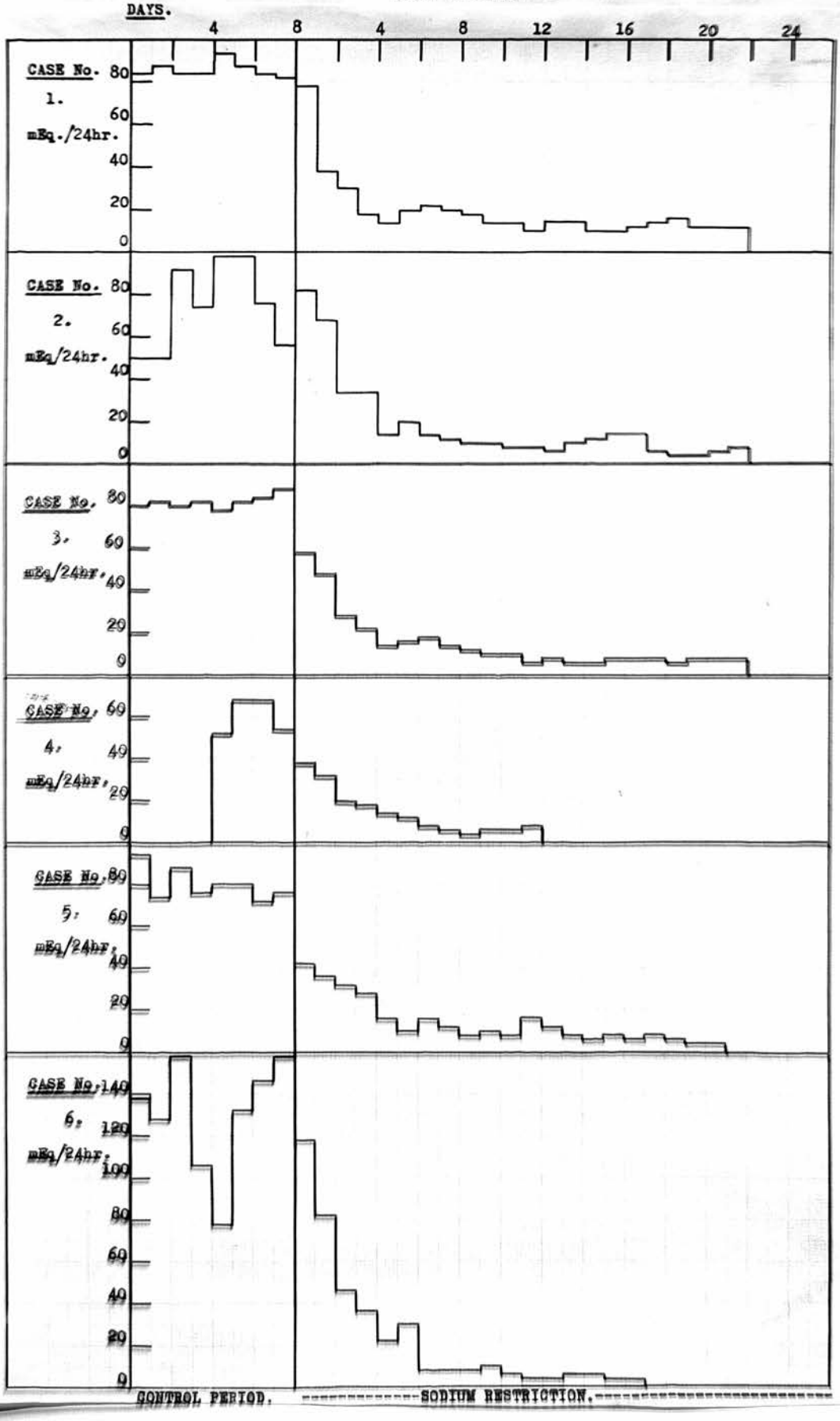


Table 6

Urinary Excretion Chloride

Hypertensive Subjects	Mean 24 hr. urinary excretion chloride prior to salt withdrawal m.eq./24 hrs.	Chloride remaining in urine in 1st 24 hrs. after salt withdrawal (% of mean before salt withdrawal)	Total Cumulative -ve chloride balance after salt withdrawal. m.eq.	No. of days take to re-establish sodium balance after salt withdrawal.
1	86.9	77.1	182	12
2	83.5	98.2	187	11
3	82.4	71.6	145	10
4	58.8	62.9	74	7
5	75.1	54.6	105	9
6	134.9	87.5	275	8
Average	86.9	75.3	161	9
Normotensive Subjects				
1	86.5	77.8	127	7
2	88.4	85.9	180	10
3	81.0	77.0	211	10
4	92.0	132.0	235	7
5	103.9	84.1	142	7

	Mean Value for Normotensive Subjects	Mean Value for Hypertensive Subjects	Probability of difference being due to chance.
Chloride remaining in urine in 1st 24 hrs. after salt withdrawal. (% of mean before salt withdrawal)	93.1±21	75.3±15	0.1
Total Cumulative -ve Cl. balance after salt withdrawal (m.Eq.)	237±147	161±70	0.2

To be significant P must be less than 0.05

remaining in the urine twenty four hours after dietary salt intake had been restricted (expressed as a percentage of the mean twenty four hour excretion during the control period), the total amount of chloride excreted in excess of intake during the period of negative balance and the number of days required to regain equilibrium between the amounts ingested and excreted. Again, no difference was noted between the behaviour of the hypertensive and normotensive subjects with regard to the time taken to re-attain balance. Average figures showed the percentage of chloride remaining in the urine twenty four hours after salt withdrawal to be slightly higher for the normal blood pressure group as was the total cumulative chloride loss during the period of negative balance when mean figures were compared. As with sodium these differences were not significant.

e. Urinary potassium excretion after dietary restriction of salt

Figures 6 and 7 show the urinary excretion of potassium for normotensive and hypertensive subjects respectively during a control period and after removal of salt from the diet. Case no. 3 of the normal group was not included in this study since excretion of potassium during the control period proved to be exceedingly

Figure 6

Normal Subjects. Urinary excretion of potassium during a control period and after removal of salt from the diet. M=mean 24 hr. potassium excretion during the control period.

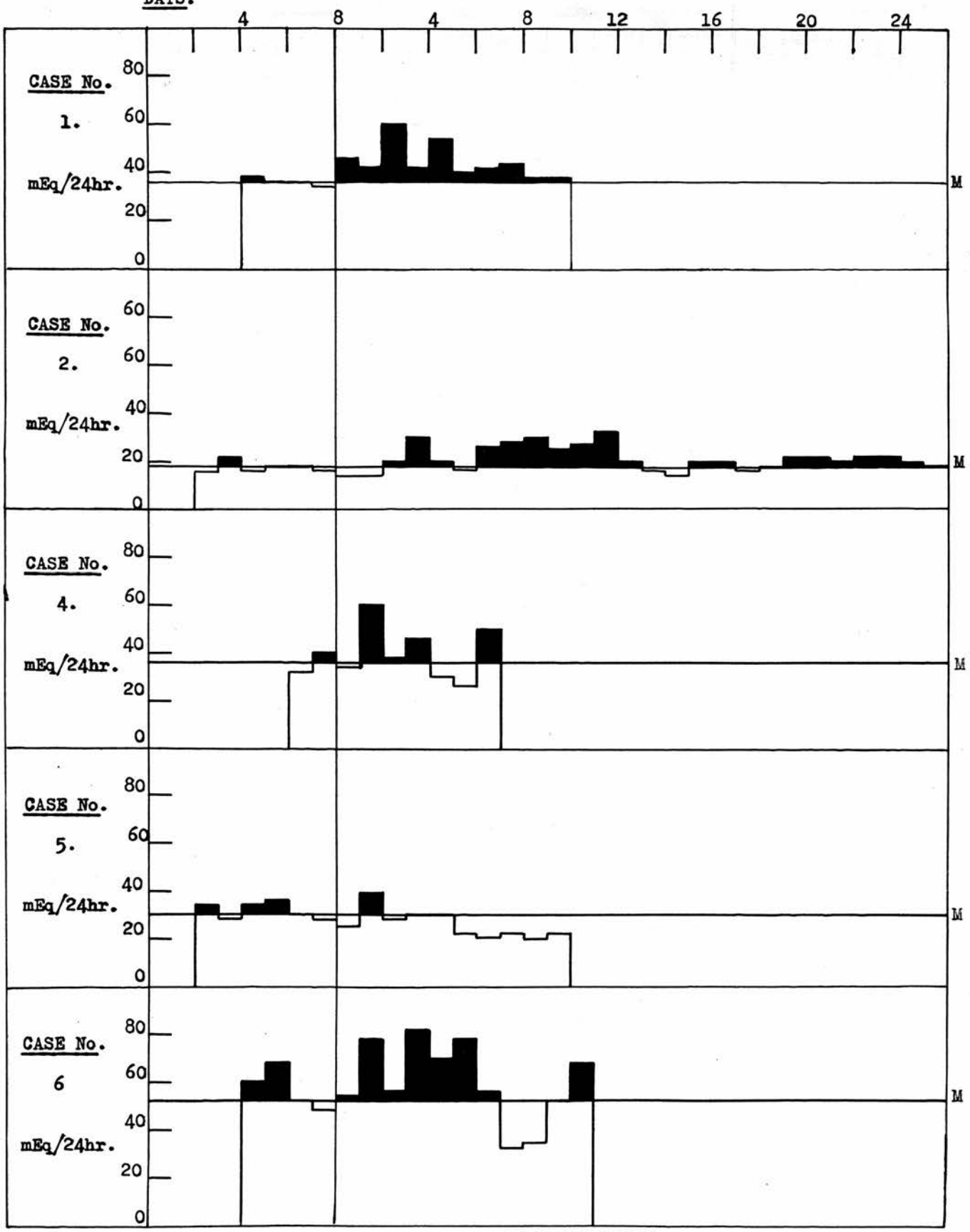
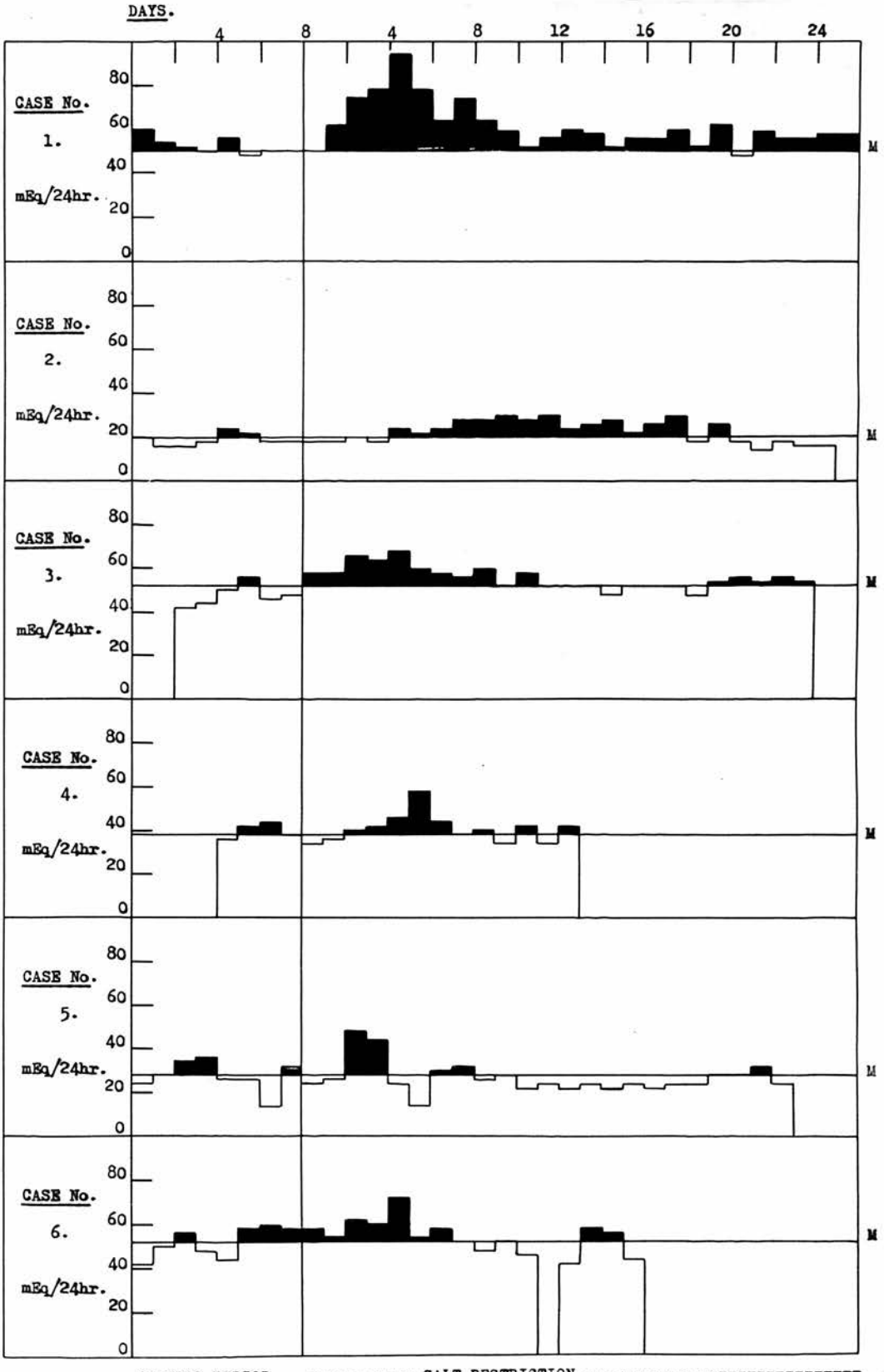


Figure 7

Hypertensive Subjects. Urinary excretion of potassium during a control period and after removal of salt from the diet. M=mean 24 hr. potassium excretion during the control period.



erratic.

In four out of the five normotensive cases excretion of potassium in the urine increased 1 to 7 days after ingestion of salt had stopped and remained elevated for periods varying from 3 to 10 days over the four subjects. Only in case no. 5 was no distinct change evident.

Urinary excretion of potassium by the hypertensive patients showed similar variations after restriction of salt intake. In each of the six cases studied a definite though sometimes slight, increase in the urinary potassium output occurred 1 to 5 days after the control period had ended and continued for periods varying from 5 to 15 days.

While the urinary excretion of potassium was high, the ion was lost from the body in excess of intake and a negative balance thus existed. The total loss of potassium during the period of negative balance varied from 8 to 113 mEq. for the normotensive subjects and from 33 to 162 mEq. for the hypertensive subjects. The potassium losses for the individual cases are listed in Table 7.

No distinct difference was noted between the behaviour of the normotensive and hypertensive subjects with regard to potassium excretion after dietary salt restriction. The magnitude of the potassium deficit was similar, on an average

TABLE 7

Urinary Excretion K

Hypertensive Subjects	Mean 24 hr. K excretion prior to salt withdrawal m. equiv.	Total cumulative K loss during -ve K balance after salt withdrawal m. eq.	Days after salt withdrawal during which there was -ve K balance
1	51	162	2-10
2	20	84	5-18
3	52	86	1-9
4	39	33	3-7
5	28	43	3-4
6	52	56	1-7
	40	77	2-9
Normotensive Subjects			
1	36	94	1-9
2	18	60	7-12
3	-	-	-
4	36	37	2-4
5	31	8	2-2
6	52	113	1-6
	35	62	3-7

	Mean Value for normotensive subjects	Mean Value for hypertensive subjects	Probability of difference being due to chance
Total Cumulative K loss during period of -ve K balance	62±42	77±46	P=.6

In order to be significant P must be less than 0.05

basis, for each group and the time, with reference to dietary salt restriction when it became evident was approximately the same.

f. Urinary nitrogen excretion after dietary restriction of salt

Figures 8 and 9 show the urinary excretion of nitrogen for the normotensive and hypertensive subjects respectively during a preliminary control period and after restriction of dietary salt.

Of the six normotensive cases, four (case nos. 1, 2, 3 & 4) showed no consistent changes in urinary nitrogen output after removal of sodium chloride from the food. One subject (no. 5) demonstrated a slight increase and one (no. 6) a slight decrease.

In the hypertensive group variations in nitrogen excretion during the period of salt deprivation were equally inconsistent. In two cases (case nos. 1 & 3) the level of nitrogen excretion was exceedingly constant throughout the entire period of study. Of the four remaining patients nos. 2 and 4 evidenced a slight increase in urinary nitrogen after withdrawal of salt from the diet while nos. 5 and 6 demonstrated no definite or consistent change.

Thus, in the particular cases used for this study the effect of salt deprivation on excretion of nitrogen in the urine was variable and no

Figure 8

Normal Subjects. Urinary excretion of nitrogen during a control period and after removal of salt from the diet. M=mean 24 hrs. nitrogen excretion during the control period.

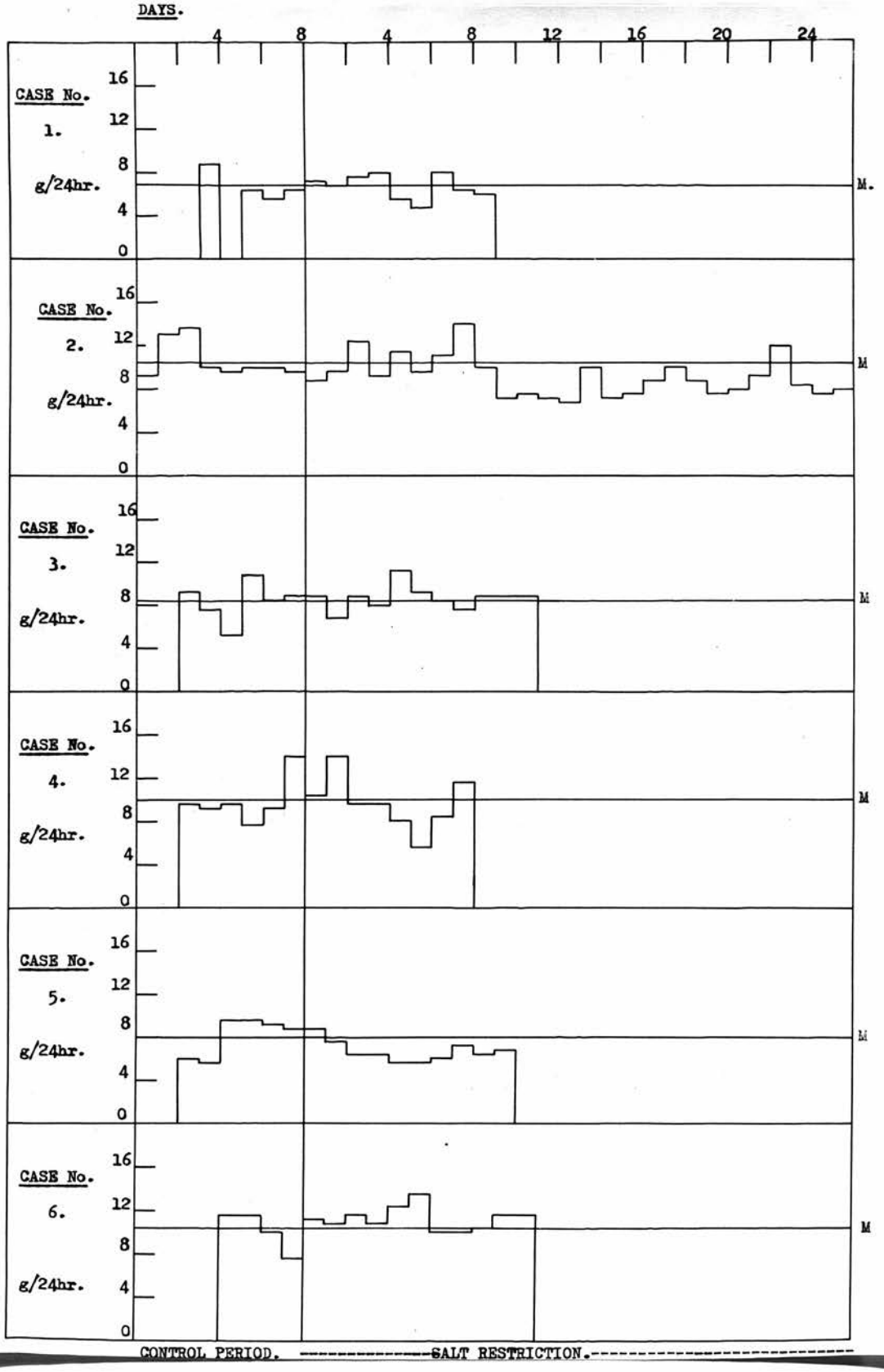


Figure 9

Hypertensive Subjects. Urinary excretion of nitrogen during a control period and after removal of salt from the diet. M=mean 24 hrs. excretion of nitrogen during the control period.

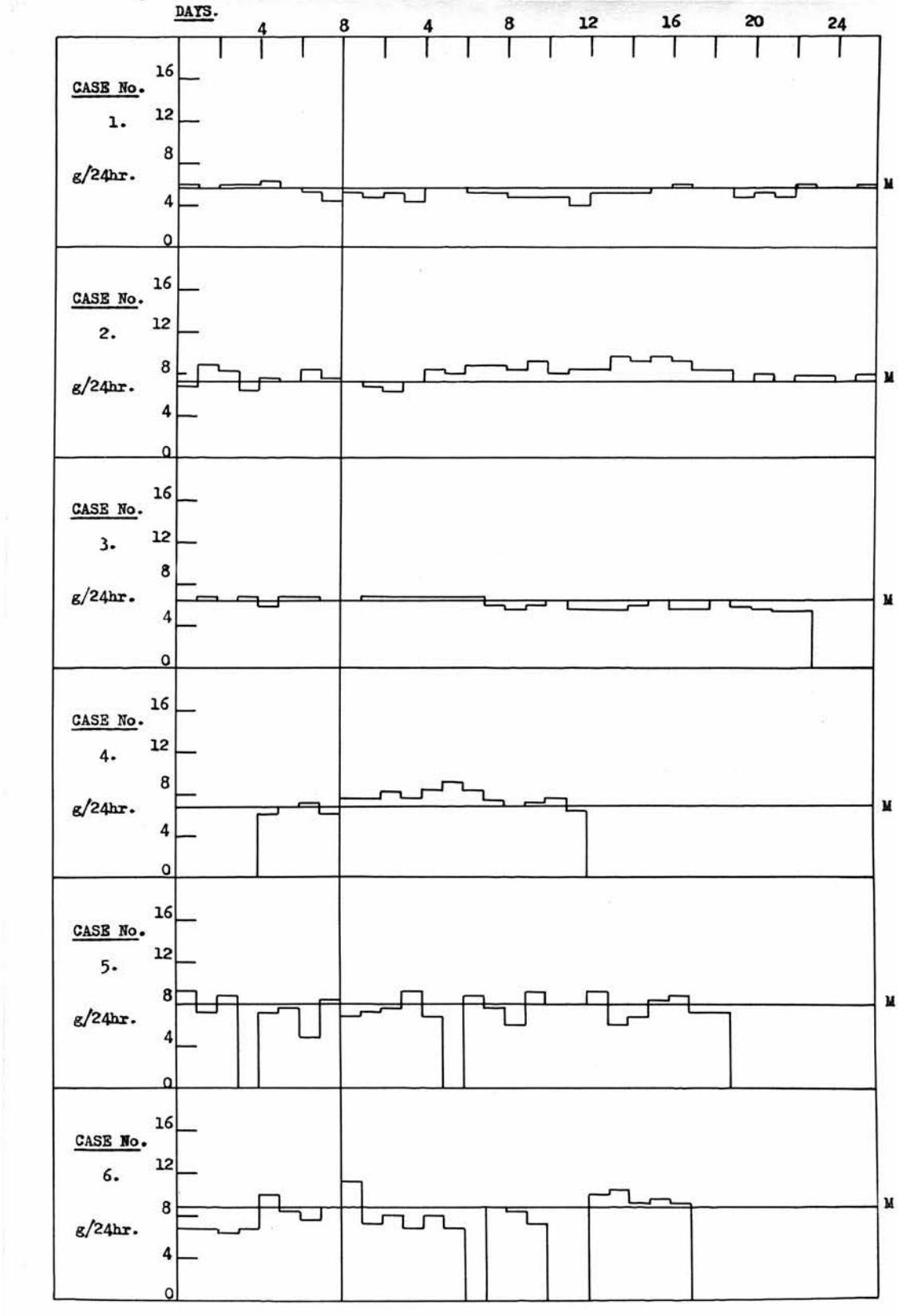


Table 8

Glomerular Filtration Rate

Hypertensive Subjects	Method used to measure G.F.R.	G.F.R. prior to salt restriction ml/min,	% change 5 days after salt restriction	% change 10 days after salt restriction	% change 20 days after salt restriction	% change 30 days after salt restriction
1	Inulin Clearance	88	-7	-	-25	-26
2	Inulin Clearance	75	+2	-3	-15	-
3	Inulin Clearance	82	-16	-10	-26	-
4	-	-	-	-	-	-
5	Inulin Clearance	88	-	-	-1	-
6	Creatinine Clearance	81	-	+16	-	-
Normotensive Subjects						
1	Creatinine Clearance	118	-23	-	-	-
2	Inulin Clearance	94	-	+7	+4	-
3	Creatinine Clearance	177	+10	-	-	-
4	Creatinine Clearance	170	+11	-12	-	-
5	Creatinine Clearance	95	+4	-21	-	-
6	Creatinine Clearance	118	+2	-21	-	-

consistent pattern of change could be observed in either the hypertensive or the normal subjects.

g. Glomerular filtration rate after dietary restriction of salt

The glomerular filtration rates for the normal and hypertensive subjects and the changes which were noted after salt had been removed from the food are shown in Table 6. Renal clearance of inulin or creatinine was used to measure this factor and the values which were obtained prior to dietary restriction of sodium chloride were within the normal ranges of the methods used for every subject studied.

During the first five days of salt deprivation, measurement of GFR was made in three cases of hypertension and in five normal cases. Changes varied from -16% to +2% for the former subjects and from -23% to +11% for the latter, thus showing no consistency in either group. After sodium chloride had been lacking in the diet for ten days there was still no evidence of a distinct increase or decrease in GFR. Three subjects from the high blood pressure group showed variations ranging from -3% to +16% and four normal persons showed changes within the limits +7% to -21%.

In the later stages of salt deprivation estimations of renal clearance were carried out in four of the hypertensive cases but in only one normal case. The latter showed an increase of 4% over the value obtained for GFR during the control period but the former subjects showed decreases ranging from 1% to 26%.

Thus, in the experiments now reported removal of salt from the diet resulted in neither definite nor consistent changes in GFR in hypertensive or normal persons.

h. Concentration of sodium, potassium and chloride in serum

Figures 10 and 11 show the serum concentrations of sodium, potassium and chloride in hypertensive and normotensive cases respectively during a preliminary control period and after removal of salt from the food. A slight fall in serum chloride was noted in three hypertensive cases (nos. 2, 4 & 5) towards the end of the period of salt deprivation. Concentrations of sodium and potassium remained constant throughout the period of study in all the experimental subjects.

6. DISCUSSION

a. Changes in weight and urine volume during the first forty eight hours of salt deprivation

Figure 10

Normal Subjects. Serum concentrations of sodium, chloride and potassium during a control period and after removal of salt from the diet. The blackened part of the histogram represents chloride concentration.

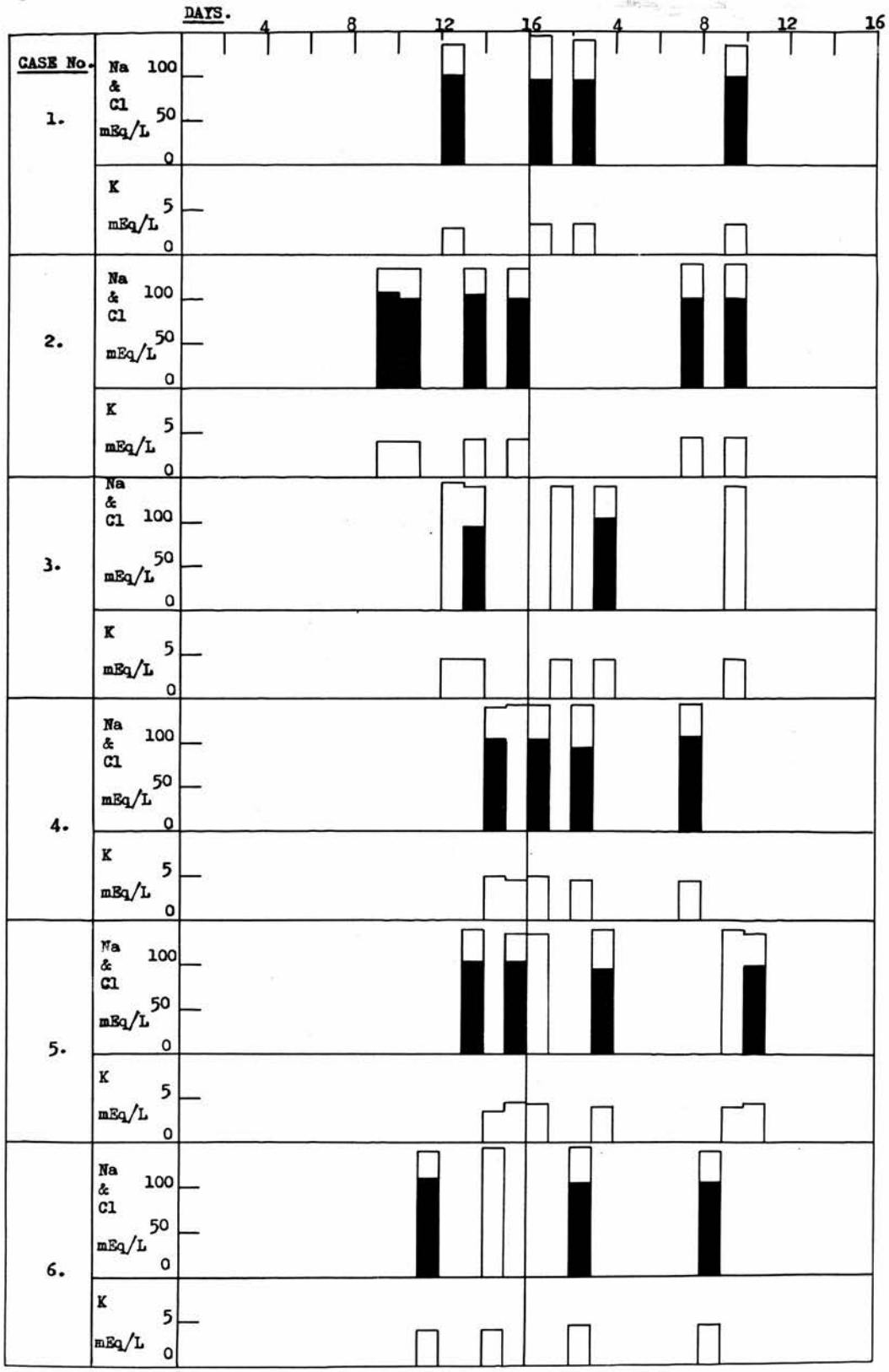
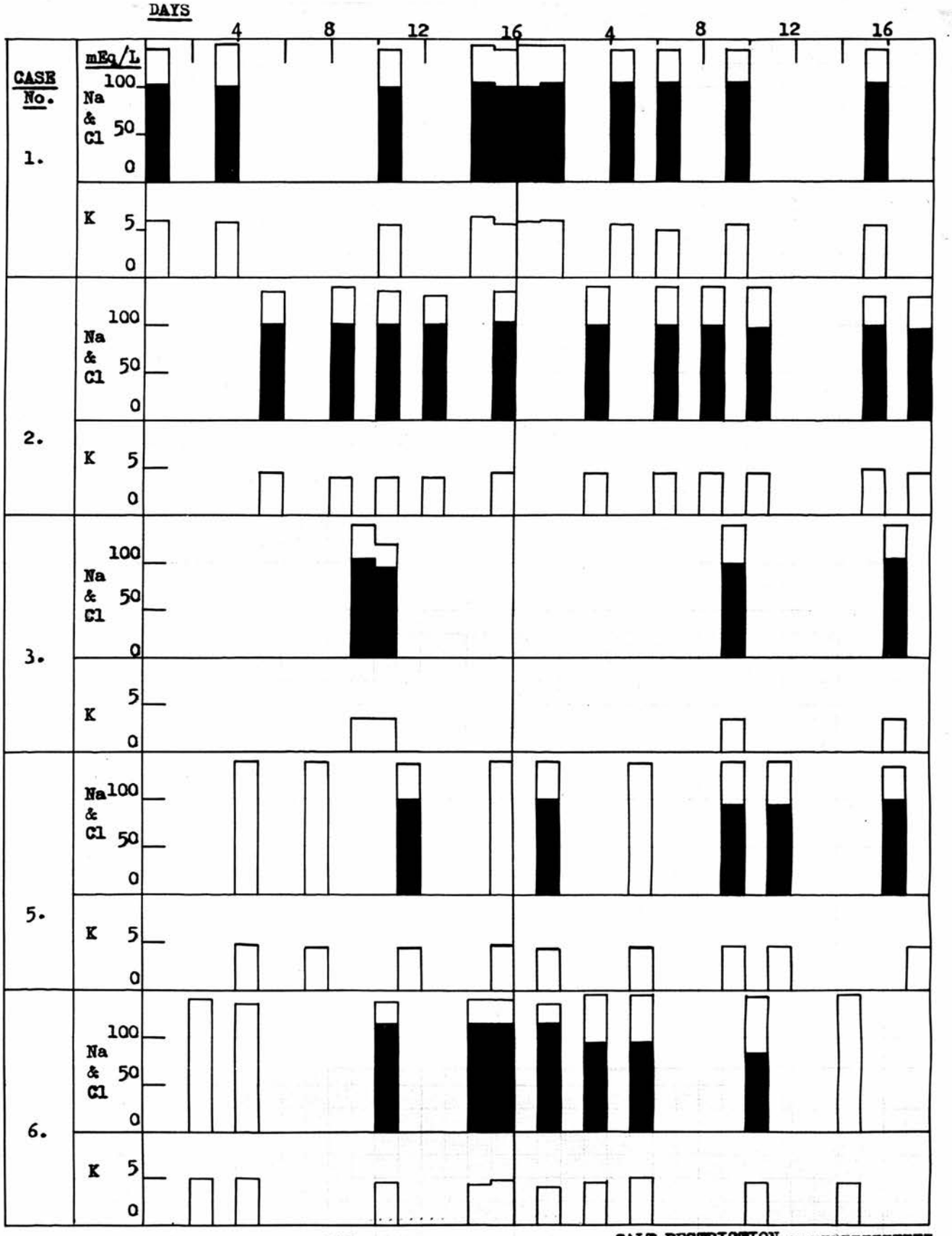


Figure 11

Hypertensive Subjects. Serum concentrations of sodium, chloride and potassium during a control period and after removal of salt from the food. The blackened part of the histogram represents chloride concentration.



In these experiments the observations which were made on changes in weight and in urine volume twenty four and forty eight hours after ingestion of salt had been abruptly stopped revealed no consistent or significant difference between the hypertensive and normotensive groups of experimental subjects.

This is in contrast to the findings of Perera and Blood (1946) who reported that, despite otherwise constant conditions sodium restriction was followed by significant weight loss and increased urine output in control subjects which was not evident in hypertensive patients. These authors found the mean weight loss of their control subjects to be 1.0 Kg. in twenty four hours as compared with 0.2 Kg. in the patients with hypertension and further, that all of the former people lost more than 0.55 Kg. whereas weight loss among the latter never exceeded 0.48 Kg.

It has already been pointed out that the difference between the mean weight losses by the two groups of subjects was found, in these experiments, to be slight and insignificant. It is of interest that only one normal person lost more than 0.55 Kg. in the first twenty four hours after salt ingestion had ceased and even after forty eight hours no control subject

showed a decrease in weight as great as 1.0 Kg. A decrease of such magnitude was only evident in one case of hypertension after two days of salt deprivation.

Similarly dietary salt restriction caused changes in urine volume which were completely parallel for the normal and hypertensive subjects of these experiments. Five out of six normotensive persons exhibited a diuresis during the first twenty four hours after removal of sodium chloride from the food and the proportion was the same in the hypertensive group. After forty eight hours of salt deprivation this diuresis was still evident in three normal cases and in five hypertensive cases. There was no significant difference in the magnitude of the increase in urine volume either after twenty four or forty eight hours of salt deprivation when mean figures for the two groups were compared.

Thus, with regard tochanges in urine volume and body weight no abnormality could be detected in the response of hypertensive subjects to abrupt and rigid restriction of dietary salt. No evidence was therefore gained which would confirm the conclusion drawn from their experiments by Perera and Blood (1946), that a disturbance in salt and water

metabolism exists in the hypertensive condition.

b. Excretion of sodium and chloride in the urine after withdrawal of dietary salt

When salt was removed from the food of six normal and six hypertensive subjects conservation of the essential supplies of sodium and chloride in the body was effected by means of a gradual decrease in the amounts of the two ions excreted in the urine until equilibrium was reached between dietary intake and urinary output. There was no distinct divergence in behaviour in this respect between those subjects suffering from hypertension and those subjects who showed normal blood pressure.

Consideration of the response of the hypertensive and normal subjects to abrupt restriction of dietary salt, with regard to the immediate reduction of sodium and chloride in the urine, the total deficits of these ions during the period of negative balance and the number of days required to re-establish equilibrium, revealed no definite variation in behaviour between the two groups. A slightly more rapid loss of sodium was noted in the normal than the hypertensive cases but no evidence was obtained which would indicate any distinct abnormality in sodium metabolism in the cases of hypertension.

Since serum sodium and chloride concentrations remained practically unchanged, at least for the first fourteen days of salt restriction, the results of these studies provide no support for the suggestion made by Perera and Blood (1946) that the renal clearance of sodium or chloride is significantly different in the two groups. Further, contrary to the observations of these workers no subject complained of symptoms suggestive of salt depletion throughout the entire experiment.

It seems most probable that the sodium and chloride which were lost in the urine in excess of intake during the period of negative balance came from the extracellular fluids of the body since, in most cases, decrease in weight and diuresis also occurred. Dole et al (1951) noted the loss of the two ions by hypertensive patients in the first few days of salt deprivation and suggested that the proportional relation between sodium and chloride excretion in the days following reduction in intake indicated that the sodium lost from the body came from a source with the composition of extracellular fluid. In an earlier paper (1950) these same workers noted that the patients for a time gained weight at a rate which was just sufficient to account for the formation of

extracellular fluid from the newly available sodium when this was once more provided in the diet.

These investigators suggested that sodium retention might be a contributory factor in causing hypertension, the metabolic disturbance concerned being changes in the degree of intracellular hydration and, if this were so, the beneficial effects of low salt therapy might be attributed to removal of the excess sodium from the body. In view of this suggestion it is of interest that, in the present study, no difference was observed between the amounts of sodium and chloride lost by hypertensive patients after salt ingestion had ceased and the quantities lost by normal subjects.

c. Glomerular filtration rate and concentration of sodium and chloride in serum

Changes noted in GFR after removal of salt from the food were variable and inconsistent.

It has frequently been reported in the literature that salt deprivation results in a decrease in GFR both in cases of hypertension and in normal persons. In general, however, the experimental conditions have been more severe than those of the present study.

McCance and Widdowson (1937) found, in

their experiments on salt deficiency that this condition produced prompt decreases in renal clearance of inulin and creatinine. The subjects of these experiments were, however, rendered salt deficient by ingestion of a diet low in sodium chloride content together with induced sweating. Nickel et al (1951) who utilised low sodium intake in conjunction with a cation exchange resin to effect salt depletion observed a decrease of 45% in the glomerular filtration rate of patients with renal insufficiency but noted no change in one normal person.

Black et al (1950) and Chasis et al (1950) reported a decrease in the glomerular filtration rates of normal and hypertensive subjects respectively on a rice-fruit diet. The effective diets were, however, lower in protein content than those used in the experiments reported here and some divergence in results would be expected.

It seems therefore that the conditions in the present study were not sufficiently severe to evoke changes of a consistent pattern in GFR. Thus, since only minor variations in the concentrations of sodium and chloride in the serum of the hypertensive and normal subjects were noted, decrease in the filtered loads of

sodium and chloride does not seem to provide a satisfactory explanation of the reduced excretion of the ions which was observed in all the experimental subjects after salt had been removed from the food. It appears to be more probable that conservation of body salt was effected by increased reabsorption of sodium and chloride by the kidney tubules.

In 1948 Wesson and his associates postulated the theory that the deciding factors in the regulation of sodium excretion are variations in serum sodium concentrations and in GFR. This would, of course, be equally hard to prove or disprove since the changes suggested are so small as to be inaccessible to available methods for measuring GFR and serum sodium, but in 1950 Black and his associates reported experimental evidence to oppose this theory. These investigators induced substantial variations in GFR by infusing hypertonic saline into healthy subjects, some on a normal diet and some on a diet of rice and fruit containing less than 0.25 mg. of sodium per day. They determined inulin clearance, plasma sodium and urinary sodium simultaneously and were thus able to compare sodium load and reabsorption by the tubules over a wide range of sodium load. They

concluded, from the results they obtained that sodium reabsorption is much higher on a salt poor diet and that changes in tubular function are mainly responsible for sodium conservation on such a diet.

d. Excretion of nitrogen in the urine after withdrawal of dietary salt

After salt was removed from the food a slight increase in urinary nitrogen excretion was observed in one normal person and in two hypertensive patients. Considering all twelve experimental subjects the response to withdrawal of salt from the diet was neither consistent nor definite.

In the literature a negative nitrogen balance observed in persons undergoing salt deprivation has been reported by several authors. As with the experiments which resulted in a definite decrease in GFR, however, the conditions have been in general more severe than those reported here.

McCance (1936) and McCance and Widdowson (1937) using induced sweating in conjunction with a low salt diet brought about depletion of salt supplies in normal persons and noted an increased urinary excretion of nitrogen. Black et al (1950) observed negative nitrogen balance in normal subjects existing on a rice-

fruit diet of lower protein content than the diet used in the present study. Leaf and Couter (1949) came to the conclusion, from their work on the effect of variation in salt intake on normal persons that sodium restriction produced negative nitrogen balance and subsequent administration of the ion restored positive nitrogen balance. The subjects of their experiments were, however, normal people living an ordinary active life as opposed to the more restful existence of patients in hospital.

A negative nitrogen balance has also been noted in hypertensive patients existing on a low salt intake. Thus, Currens et al (1949) observed that a rice-fruit diet caused increased nitrogen excretion and negative balance in two patients with hypertension. This increase, however, persisted after the addition of salt to the diet. Moreover the conditions were not entirely parallel since one of the patients had previously undergone sympathectomy.

It appears, therefore, that the conditions of the experiments just reported were not such as to produce consistent changes in nitrogen excretion by evoking catabolism of protein, and that the increased excretion of potassium which was observed in six cases of hypertension and

in four normal cases cannot be explained by release of the ion due to this process. Further, it appears reasonable to conclude that salt deprivation does not, per se, affect nitrogen balance.

e. Excretion of potassium in the urine after withdrawal of dietary salt

It was noted by Kempner (1946) that hypertensive patients undergoing treatment with the rice-fruit diet showed a higher excretion of potassium than when a normal diet was being ingested. This might, of course, have been due to the fact that the fruit content of the diet was high. Leaf and Couter (1949) concluded that restriction of salt in the food brought about an increased urinary excretion of potassium. This could, however, be explained in part at least, by the fact that these authors also observed increased excretion of nitrogen when sodium chloride was not present in the diet indicating that catabolism of protein had taken place in which case potassium ions would be released for excretion in the urine.

In all six cases of hypertension studied in the experiments now reported and in four of the five normal cases an increase in urinary excretion of potassium occurred after the cachets of sodium chloride were withdrawn from

Table 9

Hypertensive Subjects	Total cumulative Na loss after dietary salt restriction. m.eq.	Total (approx.) wt. of extra-cellular fluid. ml.	K content (approx.) of extracellular fluid. m.eq.	Total Cumulative K loss after dietary salt restriction. m.eq.	Difference m.eq.
1	162	1200	5	162	157
2	202	1500	5	84	79
3	142	1000	5	86	81
4	104	900	4	33	29
5	178	1300	6	43	37
6	164	1100	5	56	51
Normotensive Subjects					
1	61	400	1	94	93
2	188	1300	6	60	54
3	236	1600	7	-	-
4	215	1400	6	37	31
5	130	1000	4	8	4
6	396	2800	11	113	102

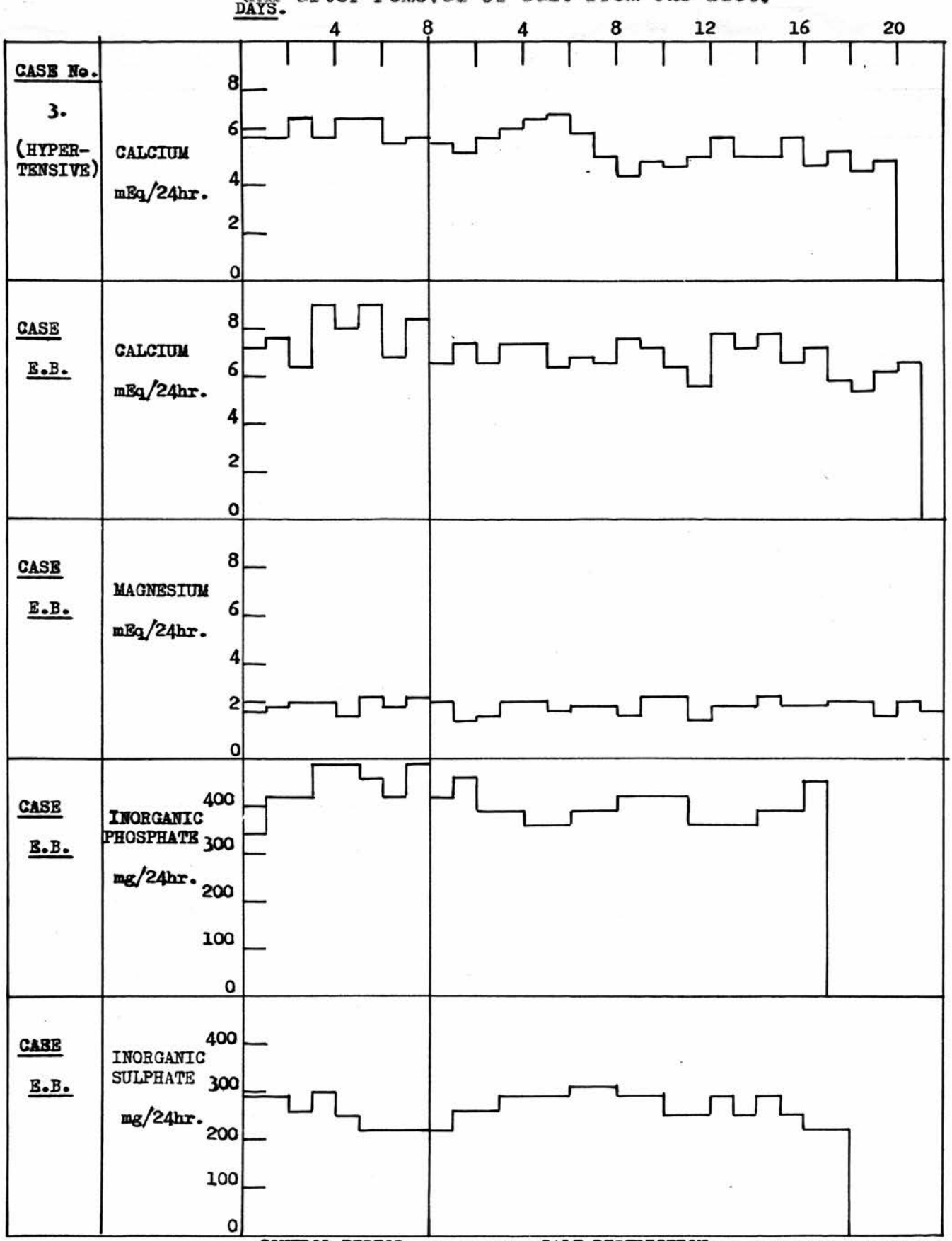
the food. This increase was temporary and continued for periods varying from three to fifteen days, no distinct difference being discernible between the response of the hypertensive subjects and that of the normal subjects to dietary salt restriction in this respect. Since all other factors in the food remained constant throughout the control period and the period of sodium chloride deprivation and all conditions of the experiment were unchanged it seems that this transient negative potassium balance must have been directly stimulated by salt restriction. That it was a secondary consequence of catabolism or failure of anabolism of protein is improbable as has already been pointed out in discussing the urinary excretion of nitrogen by the experimental subjects. Neither do the quantities excreted fit the theory that the potassium came from the extracellular fluid lost from the body in accumulating the deficit of sodium and chloride before balance was established between the low intake of salt and the urinary output. This is shown in Table 9. It appears reasonable to theorise, therefore, that the increased excretion of potassium may have furnished part of the mechanism whereby the kidney achieved conservation of body sodium and chloride in

both hypertensive and normal subjects on a salt free diet.

It was only possible to carry out estimations of urinary calcium in one of the hypertensive cases used for the rest of this study, case no. 3. For this person, however, who showed a cumulative potassium loss after salt was removed from the diet of 86 mEq., the excretion of calcium in the urine showed no distinct increase or decrease. This is illustrated in Figure 12 together with the urinary excretion of calcium, magnesium, inorganic phosphate and inorganic sulphate during a preliminary control period and after salt had been removed from the diet of a forty seven year old female with a mean systolic blood pressure of 185 mm Hg(±15) and a mean diastolic blood pressure of 108mm Hg(±7). This case (E.B.) was not included in the other studies which have been reported but it is of interest that urinary levels of calcium and magnesium showed no distinct change during the whole period of examination, nor was any variation evident in the amount of inorganic phosphate or sulphate in the urine when salt was restricted in the food. This does not, of course, prove that the amounts of these ions in the urine of the hypertensive and normal subjects studied in examining potassium excretion

Figure 12

Urinary excretion of calcium, magnesium, inorganic phosphate and inorganic sulphate by hypertensive subjects during a control period and after removal of salt from the diet.



did not vary between the control period and the period of salt deprivation. It does, however, indicate that a diet low in sodium chloride content is without effect on urinary excretion of the anions in question and causes no change in the concentration of inorganic cations, other than potassium, in the urine.

7. CONCLUSIONS

1. In six cases of hypertension carefully studied during a control period and during a period of salt deprivation there was no evidence of any disturbance in salt and water metabolism, in that the response of the hypertensive subjects to abrupt and rigid restriction of dietary salt showed no distinct variation from the response of six normal persons under identical experimental conditions.

2. Essential body supplies of salt were effectively conserved by both groups of experimental subjects by increased tubular reabsorption of the sodium and chloride ions.

3. Removal of salt from the diet consistently resulted in increased excretion of potassium in the urine. No change was noted in the excretion of other anions and cations measured in the urine after the control period had ended and dietary restriction of salt had commenced.

4. In order to study the subject fully it seemed

necessary to consider the possibility that the adrenal cortex was concerned in the process which ensured conservation of body salt when this was no longer present in the food. Section II of this report is, therefore, concerned with the role of the adrenal gland in the regulation of sodium chloride excretion and the results of an examination of the urinary corticosteriod excretion of the experimental subjects will be reported.

SECTION II - STEROIDS

PART I - GENERAL INTRODUCTION

1. THE EFFECTS OF ADRENAL INSUFFICIENCY

In adrenal insufficiency there is impairment of the mechanism whereby sodium is reabsorbed by the kidney tubules. Consequently sodium and water are lost from the body and changes in extracellular fluid ensue which are reflected, in extreme cases, in an increased serum potassium concentration and a decreased serum sodium and chloride concentration. Such effects may be observed in patients suffering from Addison's disease and in adrenalectomised animals. It is clear therefore that the adrenal cortex is concerned in regulation of these ions by the kidney.

Amongst the earliest workers to express the view that the adrenal is concerned in the metabolism of sodium and chloride were Bauman and Kurland who, in 1927, observed a fall in sodium and chloride and a rise in potassium in the plasma of an adrenalectomised cat. In the same year it was shown by Marine and Bauman that the injection of various sodium salts would increase the survival time of adrenalectomised animals and, in 1928, Rogoff and Stewart demonstrated the same effect with Ringer's Solution. The full significance of these early findings was not fully recognised, however,

until the relationship of adrenal cortical function to electrolyte metabolism was clarified by the work of Loeb (1932) who showed that a fall in plasma sodium was a characteristic of crisis in Addison's disease and went on to demonstrate that the patient could be kept symptom free by the administration of large quantities of sodium chloride. (1933). Loeb and his collaborators also showed that the fall in plasma sodium in an adrenalectomised dog was due to excessive excretion of this ion by the kidney (1933 A & B) and the link between the adrenal cortex and sodium metabolism was complete when Harrop and his co-workers (1933 and 1936) demonstrated that adequate amounts of cortical extract restored the electrolyte pattern to normal in the blood and prevented the renal wastage of sodium and chloride.

2. THE ISOLATION OF CRYSTALLINE STEROIDS FROM THE ADRENAL CORTEX

After the preparation of the first potent extracts of the adrenal cortex it was presumed that the active agent in these extracts which was responsible for maintaining life in adrenalectomised animals was a single substance - the essential hormone of the adrenal cortex. To this hormone the name Cortin was assigned. It was hard, however, to reconcile the concept of a single hormone with the isolation, during the next few years, of several crystalline steroid substances from adrenal cortical

extracts which were potent, to varying degrees, in maintaining adrenalectomised animals.

The isolation and characterization of the steroid hormones were largely carried out by four investigators - Kendall, Pfiffner and Wintersteiner in the United States and Reichstein in Switzerland. In 1936 Mason, Myers and Kendall showed their compound E, now known as cortisone to be effective in the work performance test of Ingle (1936) and, for the first time, physiological activity was clearly associated with a crystalline compound. Corticosterone was obtained in pure form in 1937 by Reichstein and his collaborators and, since this was so much more active than cortisone in the life maintenance test it was considered that corticosterone might be the principal hormone of the adrenal cortex. In the years that followed, however, a series of 29 crystalline steroids was obtained from the adrenal cortices of animals and at least six of these possessed activity in maintaining adrenalectomised animals. Moreover, it was reported by Kendall in 1940 that the amorphous fraction, the uncrystallisable residue left after the separation of the characterized steroids was still very active in this respect. As the structures of the isolated steroids were elucidated it became evident that physiological properties varied with chemical structure and the view that the adrenal cortex was represented in

physiological processes by one hormone could not be substantiated.

During the past ten years chromatographic procedures for separating small quantities of complex mixtures into their individual components have been perfected. Recently these methods have been applied to the separation of steroids from the adrenal venous blood of animals and from adrenal extracts. As a result a new and highly potent adrenal steroid has been isolated. This compound, discovered in 1952 by Tait and his associates, was provisionally termed electrocortin. Later the chemical structure was established by British and Swiss investigators working in collaboration and since an aldehyde group was present the hormone was designated aldosterone. (Simpson et al 1954).

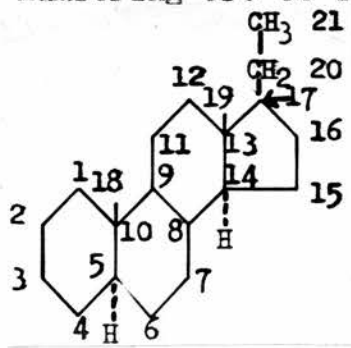
No answer has as yet been found to the question of whether all the isolated steroids are actually secreted by the gland during life.

3. THE STEROIDS OF THE ADRENAL CORTEX

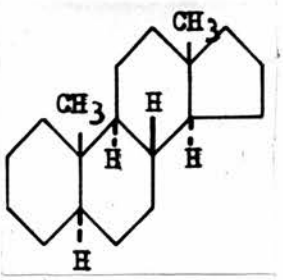
a. Structure

The saturated hydrocarbons from which the adrenal steroids are derived are allopregnane and androstane. The stereochemical formula and system of numbering are as shown.

Allopregnane



Androstane



In certain positions of the hydroxyl groups substituted in the nucleus two stereo-isomerides are possible. These have been distinguished as alpha and beta configurations, the alpha configuration being shown with a dotted valency and being regarded as below the plane of the ring concerned. The beta configuration is represented as a solid line for valency bond and regarded as above the plane of the ring. (Shoppee and Reichstein 1943).

b. Chemical Classification

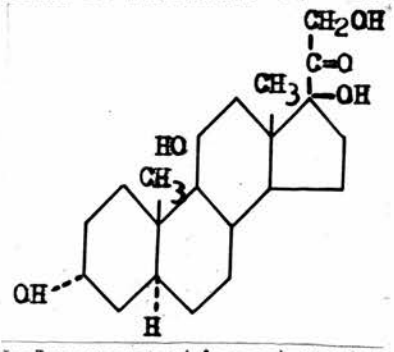
The adrenal steroids may be classified according to their carbon content. In this way two main groups are formed containing compounds with (a) 21 carbon atoms and (b) 19 carbon atoms. The constituent compounds of these groups may then be divided according to their oxygen content. Oestrone ($C_{18}H_{24}O_2$) remains outside such a classification. Considering only those compounds which are known to be biologically active, the following compounds may be assigned to each group.

C_{21} Series

I. $C_{21}O_5$

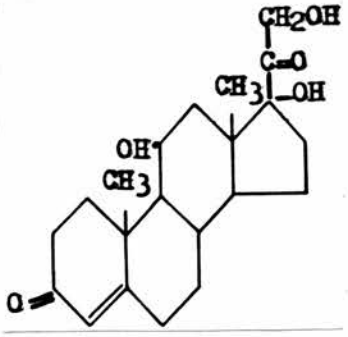
1. ALLOPREGNANE-3(α):11(β):17(β):21-TETROL-20-ONE

Reichstein & Kendall C. Wintersteiner D.



2. 17-Hydroxycorticosterone

Δ4-PREGNENE-11(β):17(β):21-TRIOL-3:20-DIONE



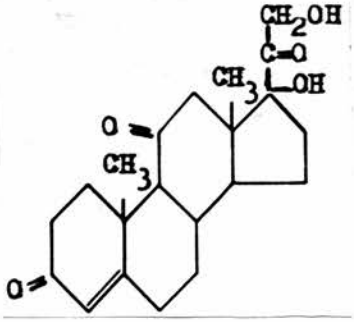
3. Cortisone

17-HYDROXY-11-DEHYDROCORTICOSTERONE

Δ 4-PREGNENE-17(β):21-DIOL-3:11-20-TRIONE

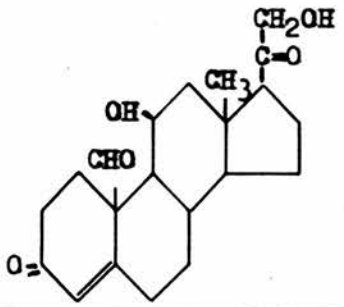
Kendall E Reichstein FA

Wintersteiner & Pfiffner F



4. Aldosterone

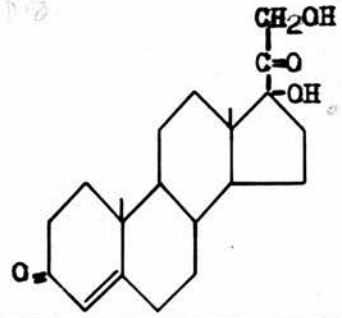
11(β):21-DIHYDROXY-3:20-DIKETO-4-PREGNENE-18-AL



II. $C_{21}O_4$

1. Δ4-PREGNENE-17(β):21-DIOL-3:20-DIONE

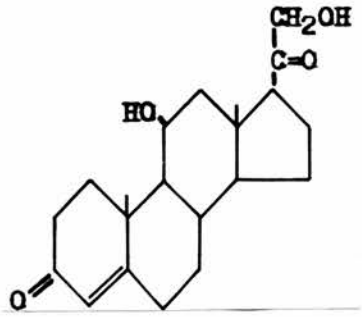
Reichstein S



2. Corticosterone

Δ 4-PREGNENE-11(β):21-DIOL-3:20-DIONE

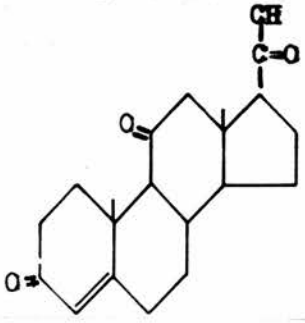
Kendall B Reichstein H



3. Dehydrocorticosterone

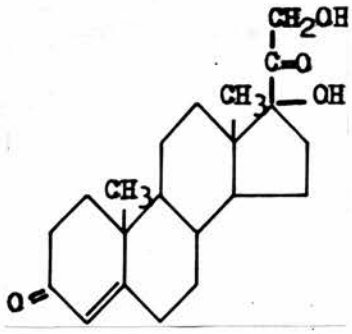
Δ 4-PREGNEN-21-OL-3:11:20-TRIONE

Kendall A



4. 17-HYDROXY-11-DESOXYCORTICOSTERONE

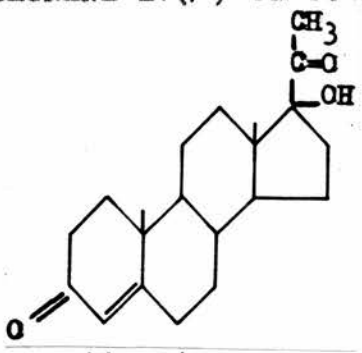
Δ4-PREGNENE-17(β):21-DIOL-3:20-DIONE



III $C_{21}O_3$

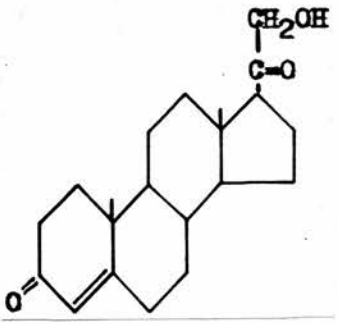
1. 17(β)-HYDROXYPROGESTERONE

Δ 4 PREGNENE-17(β)-OL-3:20-DIONE



2. Desoxycorticosterone

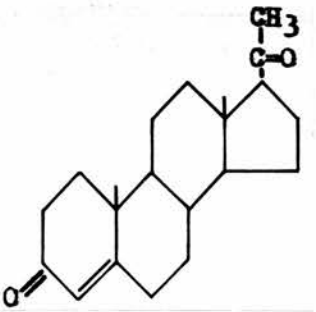
Δ4-PREGNENE-21-OL-3:20-DIONE



IV $C_{21}O_2$

1. Progesterone

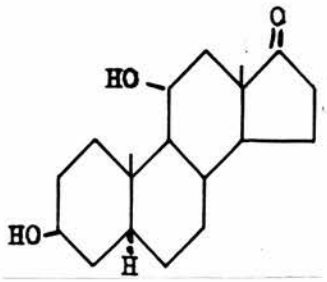
Δ4-PREGNENE-3:20-DIONE



C_{19} Series $C_{19}O_3$

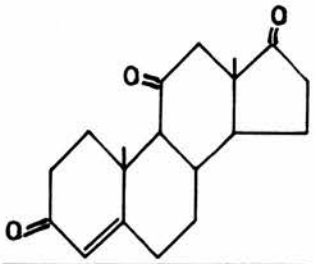
1. 11-HYDROXY-ISO-ANDROSTERONE

ANDROSTANE-3(β):11(β)-DIOL-17-ONE



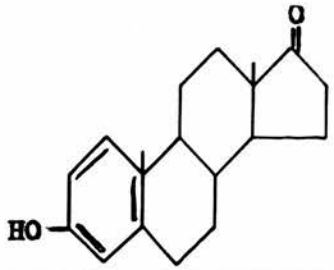
2. Androsterone

Δ4-ANDROSTENE-3:11:17-TRIONE



Oestrone $C_{18}O_2$

Δ 1:3:5:10-OESTATRIEN-3-OL-17-ONE



c. Structure in relation to biological activity

The C 4-5 double bond seems to be essential since physiological activity appears to be almost completely lost after its hydrogenation. The oxygen in the C21 position is also important since progesterone possesses much less

cortical activity than the other compounds listed in the C21 series although it was reported by Thorn and Engel in 1938 to cause retention of sodium and chloride in normal animals. Oxygen in the 17 position appears to reduce cortical activity while hydroxyl in the II has slight influence. Gluconeogenetic activity appears to depend upon the presence of oxygen at C11. The hydroxyl group at C17 increases the effect. (Hartman and Brownell 1949, p.107).

Biological tests are not, however, entirely satisfactory because of their variation and the limited amount of material available for testing.

d. Physiological Classification

A physiological classification of the adrenal steroids cannot be entirely satisfactory or clearly defined. When the compounds are placed in groups according to their major functional effects in physiological processes the constituent members of each group often overlap and exert a secondary influence in the other fields. The following classification applies to a certain extent.

1. Adrenal steroids possessing the activity of sex hormones.

2. Adrenal steroids which affect protein,

TABLE 10

Physiological Activities of Adrenal Steroids
(Young 1951)

5 = high activity 1 = slight activity

	Life Maintenance (Rat)	Renal Function (Dog)	Electrolyte Balance (Rat) (Dog)	Everse de Fremery Work Test (Rat)	Muscle Work Test (Rat)	Liver Glycogen (Rat)	Diabetogenic Test (Rat)
Corticosterone	1-2	2	1-2	1-2	3	3	3-4
17OH corticosterone	2	1	?	2	5	5	5
11-dehydrocorticosterone	1-2	2	1-2	?	2	2	3-4
Cortisone	2	1	?	2	4	4	5
11-desoxycorticosterone	4	4	5	4	1	1	1
17OH-11-desoxycorticosterone	3	4	2	2	1	1	1

carbohydrate and uric acid metabolism and also produce important haematologic changes.

3. Adrenal steroids which act mainly on sodium, potassium and chloride metabolism, and therefore on water metabolism as well.

The adrenal steroids which have been most studied are corticosterone, 17-hydroxycorticosterone, cortisone, 11-desoxycorticosterone and 17-hydroxy-11-desoxycorticosterone. The physiological activities of these compounds are listed in Table 10.

4. THE SODIUM FACTOR

Unfractionated adrenal extract will maintain plasma sodium at normal levels in adrenalectomised animals. If injected in sufficient quantities it will also cause sodium retention in normal animals. It has been shown that this extract can be divided into two fractions, one of which is responsible for sodium retention and one which will maintain adrenalectomised animals in good health. (Hartman et al 1939) The factor which is responsible for sodium retention is named, in the literature, "the sodium factor" or "the salt and water hormone".

Until very recently it was an impossible task to identify the sodium factor, by its properties, with one of the crystalline steroids isolated from the adrenal cortex. The most active compound known

in causing sodium retention was desoxycorticosterone and this can only be obtained from adrenal tissue in very small amounts. Reichstein and von Euw obtained 29 mg. desoxycorticosterone per 100 g. tissue and, in order to account for the sodium retaining properties of the adrenal, many times this amount would be required.

The discovery of aldosterone has shed new light on the problem. It has been shown that this compound has the ability to increase both sodium retention and potassium excretion by the kidney and that it is many times more active than desoxycorticosterone in this respect. (Simpson et al 1953) Further, a compound identical with this hormone in all its properties has been detected in extracts of human urine which have proved active in causing sodium retention. (Cope and Garcia-Llaurado 1954)

5. LIBERATION OF ADRENAL CORTICAL HORMONES

There are three possible ways in which the adrenal cortex might influence the regulation of body sodium content by the kidney:

1. In 1948 Harris suggested that the liberation of steroids from the adrenal cortex is controlled by the nervous system. Stimulation of centres in the hypothalamus leads to the liberation of humoral transmittors from the infundibulum and these transmittors are carried by the hypophysioportal system to the anterior

lobe of the pituitary where liberation of adrenocorticotropic hormone (ACTH) is stimulated. ACTH is carried by the blood stream to the adrenal cortex where it promotes secretion of one or more of the active hormones.

The adrenal hormone which is concerned with sodium metabolism may be under the control of ACTH and it is certainly true that the administration of ACTH to human subjects has frequently resulted in sodium retention. (Conn et al 1951 A, Forsham et al 1951) On the other hand hypophysectomised animals do not show the changes in electrolyte balance which are characteristic of adrenalectomised animals. (Ingle and Baker 1953)

2. The adrenal salt hormone may be completely independent of ACTH and its secretion may be due to a mechanism as yet unknown.

In 1950 Daughaday and MacBryde, studying urinary steroid excretion during sodium and chloride deprivation came to the conclusion that if maximal tubular reabsorption of sodium depends on adrenal salt hormone its release is independent of ACTH and is not measured by existing methods.

3. The regulation of body sodium, chloride and potassium may devolve upon the kidney which, in order to fulfil this function, must be provided

with adrenal cortical activity.

6. INTERRELATION OF ADRENAL CORTICAL HORMONES AND POSTERIOR PITUITARY HORMONES

There is general agreement as to the existence of substances which promote reabsorption of sodium and chloride by the kidney tubule. Evidence is conflicting, however, regarding the degree of importance which should be attached to these compounds as regulators of renal tubular activity.

The metabolism of these ions and water is certainly not solely controlled by the hormones of the adrenal cortex because a number of diseases other than those of the adrenal glands are characterized by disturbances in the regulation of water and electrolytes. (Overman 1951) There is some experimental evidence to show that posterior pituitary extracts may increase the urinary excretion of sodium and chloride while decreasing the excretion of water. (Roemmelt et al 1949, Gaunt et al 1949)

In 1939 Corey, Silvette and Briton first proposed the theory of physiological antagonism between the adrenal cortex and the posterior pituitary. Gaunt and Birnie have recently reviewed the arguments for this concept (1951). It is highly probable that the alterations in sodium, chloride and potassium metabolism seen in adrenal insufficiency are not due entirely to lack of cortical hormone but, in part, to an unantagonised activity of the hormones of the

pituitary glad.

7. THE PROBLEM

While relatively little information has been gained concerning the individual fates of the many steroids which have been isolated in crystalline state from the adrenal glad, change in cortical function has been clearly associated with changes in the excretion of various steroid metabolites.

Modern research work has closely allied problems of electrolyte metabolism with adrenal cortical activity and it was considered that estimation of these excreted steroid metabolites, presumed to be of adrenal cortical origin, in the cases used for study of sodium and chloride balance would afford a means of gaining further information on the complex question of their role in metabolic processes.

The first problem which had to be dealt with was that of finding a suitable method for the chemical assay of steroid metabolites which was reasonably quick, accurate and applicable to the routine procedure of daily urine analysis. This question forms the subject matter of Part II of the work which follows.

Part III deals with the urinary excretion of steroids by hypertensive and normal persons on a salt free diet and is related to electrolyte excretions which have previously been discussed.

SECTION II - STEROIDS

PART II - METHODS

1. INTRODUCTION

Methods for the estimation of corticosteroids fall into two main groups, A - Biological assays and B - Chemical assays based on the structure of the side chain (carbon atoms 17, 20 and 21).

Within the last three years paper chromatographic techniques for separating mixtures of corticosteroids in urine have been described. (Burton et al 1951, Bush et al 1952) The individual steroids may then be studied and estimated but these methods are not as yet sufficiently developed to make them useful in routine work.

a. Biological Assays

Some of the earliest methods of estimating adrenal cortical steroids were biological assays. Thus Anderson and Haymaker (1938) and Weile and Browne (1939) presented methods which involved the use of urinary extracts on adrenalectomised animals, the former workers being concerned with the ability of the extracts to maintain life and the latter considering their efficacy in affording protection against cold. In 1943 Venning et al proposed another method of bio assay utilising the ability of urinary extracts to promote the deposition of glycogen in the

livers of fasting adrenalectomised rats.

Biological methods are both efficacious and exceedingly important for experimental study of substances showing adrenal activity. Their application is, however, both tedious and time consuming and, moreover, it is probable that the substances present in urine which show cortical activity by biological tests represent only a fraction of the total urinary corticosteroids.

b. Chemical methods based on the structure of the side chain

Steroids isolated from the adrenal cortex are characterized by a particular mode of substitution of the side chain. The types of substitution which have so far been encountered are:

1. 20:21-diol
2. 20:21-ketol
3. 17:20:21-triol
4. 17:21-diol-20-one (dihydroxyacetone)
5. 17:20-diol
6. 17:20-ketol

It is reasonable to assume that urinary steroids possessing one of these side chains are of adreno cortical origin.

Well established methods of estimating

corticosteroids which are specific with pure solutions of the adrenal steroids known to occur in urine are based on the oxidative fission of the side chain with periodic acid followed by quantitative estimation of one of the end products of the reaction. The nature of the end products depends on the original side chain structure and the existing methods may be classified under three headings:

i 17 ketogenic steroids. Side chains 3 and 5 above. Converted to 17 ketosteroids by periodic acid oxidation.

ii acetaldehydogenic steroids. Side chain 5 above. Liberate acetaldehyde on oxidation with periodic acid.

iii formaldehydogenic steroids. Side chains 1, 2, 3 and 4 above. Liberate formaldehyde on oxidation with periodic acid.

In 1952 Brooks and Norymberski suggested that by substituting sodium bismuthate for periodic acid the estimation of 17 ketogenic steroids could be extended to include those steroids with a dihydroxyacetone side chain and in 1953 Norymberski and Stubbs described a method for estimating 17 ketogenic steroids utilising sodium bismuthate as the oxidising agent. They concluded that this method measured steroids bearing the side chains

17:20:21-triol, dihydroxyacetone and 17:20-diol.

Recently Brooks and Norymberski (1953) have suggested that utilisation of sodium bismuthate for selective fission of the corticosteroid side chain permits the combined estimation of formaldehydogenic and 17 ketogenic steroids and affords a simple means of differentiating and estimating three groups of adrenal steroids:

1. Formaldehydogenic, non-ketogenic.
2. Formaldehydogenic and ketogenic.
3. Ketogenic, non-formaldehydogenic.

c. Formaldehydogenic steroids

Assay of urinary corticosteroids in the experiments reported here was carried out by estimation of formaldehyde liberated after periodic acid oxidation. These methods will, therefore, be considered in more detail.

Formaldehyde can be estimated after reaction with chromotropic acid. The reaction between these two compounds was discovered by Eegriwe in 1927. Methanol, acetaldehyde and formic acid do not react with chromotropic acid in the same way and do not interfere with the estimation of formaldehyde even when present in the ratio of 10:1. The procedure consists essentially in mixing the solution containing formaldehyde with chromotropic acid in sulphuric

acid and heating the solution in a boiling water bath. Its application to the estimation of formaldehyde in biological fluids has been described by MacFadyen (1945).

In 1946 Lowenstein et al suggested a method for the estimation of corticosteroids in urine based on a quantitative estimation of the formaldehyde liberated on oxidation of urinary extracts with periodic acid. This was modified by Daughaday et al in 1948 (A) by the introduction of a distillation step whereby the formaldehyde liberated was distilled into a sulphite solution to minimise the effects of interfering substances present in small amounts in adrenal cortical extracts and large amounts in urine. The method consisted of dividing a chloroform extract of the urine between benzene and water whereupon the larger part of the corticosteroids passed into the aqueous phase which was then submitted to the periodic acid oxidation procedure. In 1950 Lloyd and Lobotski introduced a method which was essentially that of Daughaday et al but which also measured the formaldehyde liberated by periodic acid oxidation in a solvent permitting solution of steroids poorly soluble in water, such as desoxycorticosterone. A similar procedure has been suggested by Corcoran and Page (1948) who

discarded the benzene water partition on the grounds that the water extraction of certain steroids is incomplete and because the benzene water partition of adrenal cortical extracts yields a colour density in water as compared with water of about 0.65.

These methods all suffer from the disadvantage of measuring only free steroids or, at best, a small proportion of conjugated steroids. Tompsett (1953) modified the periodic acid reaction still further in applying it to the estimation of urinary corticosteroids.

The author observed that the side chain $-\overset{H}{\underset{|}{C}}-CO.CH_2OH$ such as is found in corticosterone and desoxycorticosterone was stable to the action of hot dilute mineral acids and therefore the ability of these steroids to produce the theoretical yield of formaldehyde when treated with periodic acid was unimpaired by previous treatment with such acids. It was found that the side chain attached to carbon atom no. 17 which is characteristic of cortisone, i.e. $-\overset{OH}{\underset{|}{C}}-CO-CH_2OH$ was not stable during acid hydrolysis and the latter process would, therefore, exclude steroid metabolites with a similar type of side chain structure to cortisone and 17 hydroxy corticosterone. Tompsett further pointed out

that the side chain -CH-CHOH.CH_2OH might conceivably be encountered and expressed the view that this might also be stable to hydrolysis by acids. The method was therefore described as being efficacious in measuring the urinary excretion of steroids with a side chain structure similar to corticosterone and desoxycorticosterone.

In 1954 Tompsett and Smith published the results of experiments carried out to determine the possible interference, if any, of fatty residues that may be present in urinary extracts. They found that if the results were based on the first nine ml. distillate collected the effects of non specific fatty matter such as lecithin or tri-olein was minimised.

2. CHOICE OF METHOD

The method described by Tompsett for the estimation of free and conjugated steroids of the acid stable formaldehydogenic type was used in the studies reported in this paper. It is relatively simple and suited to routine work and although it measures only a fraction of the total urinary corticosteroid content, this criticism may be levelled at all existing chemical methods for the assay of such steroid metabolites.

The accurate determination of individual adrenal cortical steroids in urine and even of closely

related groups of these compounds is dependent at the present time on chromatographic separation of the constituents of the extract and subsequent biological assay of the particular fraction or fractions concerned. Despite this fact, however, a chemical procedure may still provide a useful index of adrenal action if it can be shown that certain circumstances which produce variations in secretory activity, produce corresponding variations in the urinary steroid fraction estimated. Such an index is not, of course, entirely satisfactory since it will reflect changes in the secretion of some hormones and their metabolites but not of others. Moreover very slight changes in adrenal activity may remain undetected. Nevertheless, if it is found that the index is altered by variation in adrenal action or by administration of steroid hormones but otherwise remains constant, the chemical procedure affords a convenient means of studying the action of the adrenal gland under conditions where altered secretory activity might be expected.

Tompsett (1953) has found that the amount of acid stable formaldehydogenic steroids (ASFS) excreted in the urine of one individual does not vary greatly from day to day and further that the daily output of ASFS is lower than normal in Addisons disease and in hypopituitarism and higher than normal in cases of hyperadrenalism. The author

has also demonstrated that the excretion of ASFS is increased after administration of ACTH and the same effect has been observed during the period following surgical operation when increased adrenal activity is indicated by disturbed water and electrolyte metabolism. (Wilkinson et al 1953)

In view of these facts it seemed that the method described by Tompsett might be effective in providing some information on the problem of adrenal cortical activity in normal and hypertensive subjects existing on a diet of exceedingly low salt content. Before the procedure was applied to this question preliminary experiments were carried out on the method. The results of these experiments are reported in the following pages.

3. PROCEDURE

Experiments were carried out to examine the method described by Tompsett (1953) for the estimation of acid stable formaldehydogenic steroids (ASFS) in urine. In the early studies the method used employed oxidation with periodate at boiling point. Later this was modified, at Dr. Tompsett's suggestion, and oxidation took place at room temperature.

These experiments may be divided into four groups.

1. Examination of excretion of ASFS in the urine of normal persons in order to compare

values obtained by hot and cold oxidation methods.

2. Study of the recovery of pure steroids added to water and urine by the hot and cold methods of oxidation.

3. Studies on the recovery of pure steroids administered to patients, employing oxidation both at boiling point and at room temperature in the method of estimation.

4. Experiments to determine whether variation in adrenal activity produced by administration of ACTH to patients could be measured when ASFS or 17-ketosteroids in urine were used as the indices of adrenal action.

4. DETAILS OF METHODS USED

a. Estimation of acid stable formaldehydogenic steroids in urine

Reagents:

A.R. Chloroform

Pure absolure alcohol

A.R. Sulphuric acid

A.R. Hydrochloric acid

15 M Sulphuric acid

13 M Sulphuric acid

10 M Sulphuric acid

1% W/V Sodium sulphite ($Na_2SO_3 7H_2O$) in water

10% W/V Stannous chloride ($SnCl_2 2H_2O$) in 10% V/V hydrochloric acid.

2N sodium hydroxide

A.R. anhydrous sodium sulphate

Periodic acid reagent:

0.01 M potassium periodate in 0.15 M sulphuric acid.

Chromotropic acid reagent:

300 mg. chromotropic acid (Hopkins Cole) dissolved in 2 ml. water and made up to 50 ml. with 13 M sulphuric acid. Freshly prepared for each determination.

Preparation of pure absolute alcohol:

10 g. m-phenylenediamine hydrochloride were added to 2000 ml. absolute alcohol. The mixture was shaken and left in the dark for a period of seven days, during which time it was shaken intermittently. The alcohol was then distilled from the solution. About 300 ml. were discarded at the beginning and the end of the distillation. The purified alcohol was stored in a brown bottle.

i. Oxidation with periodic acid at boiling point

To 100 ml. urine in a 250 ml. flask with a ground glass neck were added 20 ml. concentrated HCL and the solution was boiled under a reflux condenser for 10 minutes. After cooling, the hydrolysed urine was extracted three times with 50 ml. quantities of chloroform. The chloroform extract was then washed once with 25 ml. 2N sodium hydroxide and twice with

25 ml. quantities of water. Finally it was dried over anhydrous sodium sulphate and filtered. The extract was reduced to dryness in an all-glass still on a boiling water bath and the last traces of solvent were removed by application of a vacuum pump.

The residue was dissolved in 1 ml. of absolute alcohol and transferred to a 50 ml. Kjeldahl flask with water washings (8.5 ml.). 5 ml. periodic acid reagent and 0.5 ml. concentrated sulphuric acid were added and the mixture was distilled. The first nine ml. distillate were collected in a 10 ml. graduated cylinder containing 1 ml. 1% sodium sulphite. The cylinder was stoppered and the contents mixed by inversion.

Into a tube fitted with a ground glass stopper were pipetted 2 ml. distilled water, 1 ml. distillate and 5 ml. chromotropic acid reagent. The contents of the tube were mixed and then the stoppered tube was heated in a boiling water bath for 30 minutes. After cooling, 2 ml. 10 M sulphuric acid were added and the contents of the tube mixed by inversion.

A blank determination was carried out by substituting 100 ml. distilled water for urine and submitting it to the whole process.

The optical density of the solution was

determined against the blank at 570 mμ in a Unicam S.P. 600 spectrophotometer. The concentrations of ASFS were obtained from a graph prepared by submitting standard alcoholic solutions of pure desoxycorticosterone acetate to the whole process.

ii. Oxidation with periodic acid at room temperature

The method was exactly the same as (i) until the dried extract had been transferred with washings to the Kjeldahl flask. (Volume of washings = 7.5 ml.) then 5 ml. periodic acid reagent were added and the mixture was left at room temperature for 12 - 18 hours when 1 ml. 6% stannous chloride was added followed by 0.5 ml. sulphuric acid. Distillation and colour development were carried out as before.

b. Estimation of 17-ketosteroids in urine

17-ketosteroids were estimated according to the method of Callow and his associates (1939). Readings were made at 520mμ and 480mμ on a Unicam S.P. 600 spectrophotometer and a correction factor applied to minimise the effects of interfering substances. Values were obtained from a graph prepared from standard solutions of dehydro-iso-androsterone.

5. RESULTS

a. Excretion of ASFS in the urine of normal persons

TABLE 11

ASFS in the urine of normal persons

Amounts excreted in 24 hr. estimated by hot and cold oxidation methods.

Hot Method				Cold Method			
Subject	Sex	No. of consecutive days on which ASFS was estimated	Average result mg/24 hr.	Subject	Sex	No. of consecutive days on which ASFS was estimated	Average result mg/24 hr.
R.R.	F	5	7.4	R.R.	F	1	3.9
V.F.	F	2	5.6	R.	M	6	3.8
A.C.	F	1	5.3	J.D.	M	3	3.7
B.W.	F	1	5.5	N.	F	2	2.3
J.O.	M	9	7.7	J.O.	M	1	4.2
				G.	M	4	3.0
		Average	6.3			Average	3.5

Table 11 shows the amount of ASFS excreted in 24 hours by certain normal persons. In five cases estimations were carried out using the hot oxidation procedure and in six cases oxidation at room temperature was employed. It was found that the values obtained were consistently higher when the method used to measure ASFS employed oxidation at boiling point. The comparison is not entirely satisfactory since the sex distribution was not the same in each group but two cases (J.O. and R.R.), one male and one female, are represented in each and clearly illustrate the relationship between the results obtained by the two methods.

Results for any one person were reasonably constant from day to day regardless of the temperature of oxidation used in the estimation of ASFS.

b. Recovery of pure steroids added to water and urine

In Table 12 are shown the recovery figures for pure cortisone and desoxycorticosterone added to water and for the same compounds and desoxycorticosterone acetate added to urine.

The experiments were carried out using both oxidation procedures and it was found that the temperature of oxidation did not affect the recovery of any of these steroids to a great

TABLE 12

Recovery of Pure Steroids added to water and urine by the hot and cold oxidation methods.

		Hot Periodate Method % recovery	Cold Periodate Method % recovery
Water	Desoxycorticosterone	102	100
	Cortisone	63	40
Urine	Desoxycorticosterone	82	89
	Desoxycorticosterone acetate	93	90
	Cortisone	35	40

extent. Recoveries from water were more complete than those from urine in each case and figures for desoxycorticosterone and its acetate were higher than the corresponding values for cortisone.

c. Recovery of cortisone administered to patients

The effects of cortisone on the urinary excretion of ASFS in patients suffering from various diseases and in one normal male are shown in Figure 13. In only one subject did cortisone cause a distinct rise in the amount of ASFS in the urine and this patient had received a DOCA implant seven months previously which was still active in view of the elevated blood pressure. Despite the increased steroid excretion the recovery of cortisone was less than 10% of the total dose administered.

A slight increase in urinary levels of ASFS for one day after the commencement of cortisone administration was observed in case 3 but this only represented a recovery of 2.5% of the quantity given that day. The total recovery for this patient only amounted to 1% over the whole period of cortisone administration.

Although the increase in urinary steroid levels shown by the rheumatoid arthritis patient (case no.6) persisted for two days

Figure 13

Effect of cortisone on urinary excretion of acid stable formaldehydogenic steroids (expressed as mg. desoxycorticosterone)

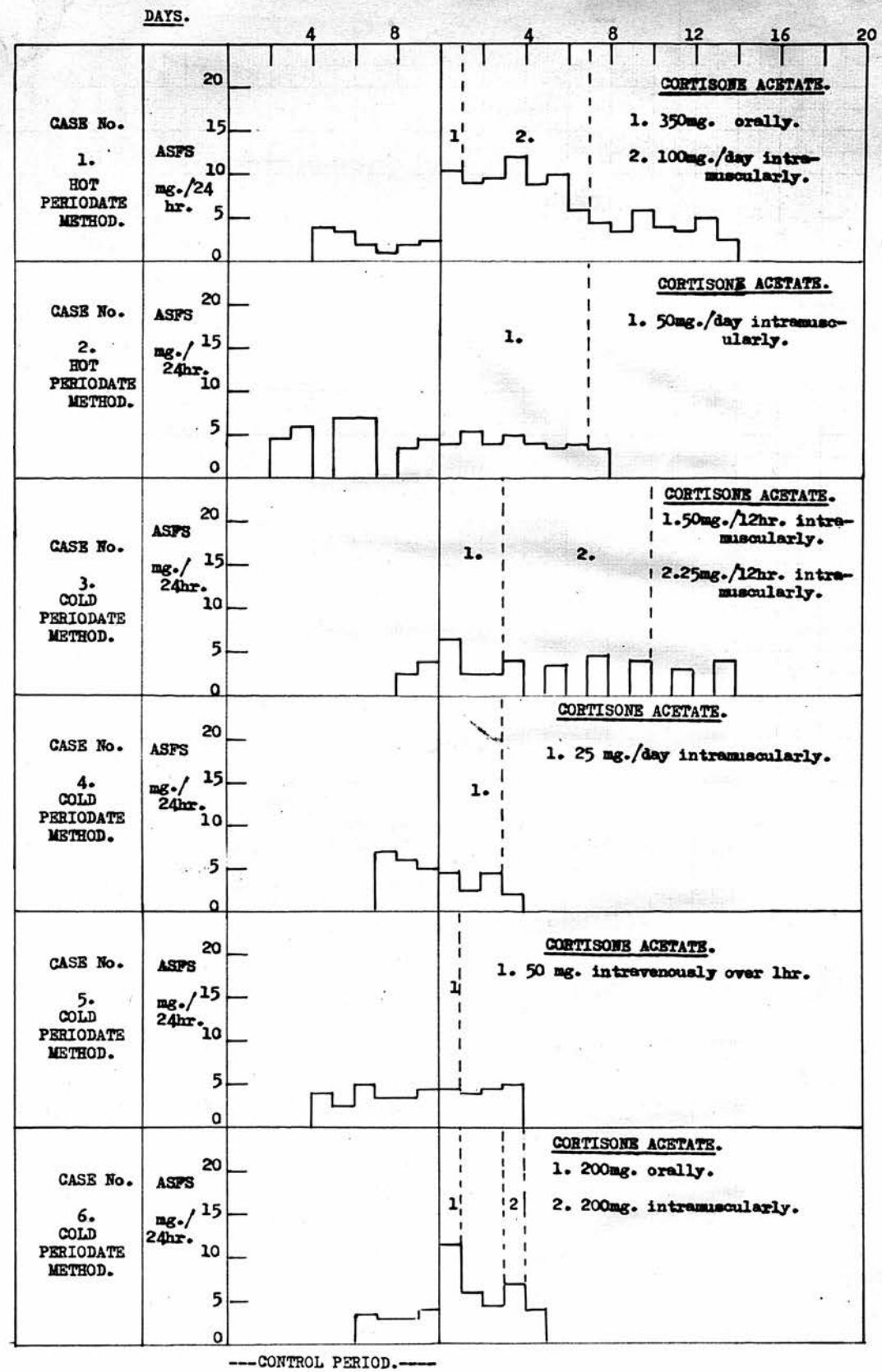


TABLE 13

Recovery of Cortisone in the Urine after administration to patients

Case No.	Sex	Diagnosis	Temp. of Oxidation	Amount of Cortisone administered mg.	Recovery %
1	M	Addisons disease	Hot	950	7
2	M	Addisons disease	Hot	350	0
3	F	Normal	Cold	650	1
4	F	Normal	Cold	75	0
5	M	Normal	Cold	50	6
6	M	Rheumatoid Arthritis	Cold	200	a) orally - 6 b) intramuscularly - 2

after the oral administration of cortisone the total increase was equal to less than 10% of the amount received.

The recovery figures for each case are shown in Table 13. The temperature of oxidation used in the estimation of ASFS did not affect these figures.

d. Recovery of desoxycorticosterone glucoside administered to a normal subject

Figure 14 shows the effects of desoxycorticosterone glucoside on urinary excretion of ASFS by a normal male: (a) for 110 minutes after injection, and (b) for the two twelve hour periods immediately following injection. The increase is evident.

4 ml. of a 1000 mg% solution of the glucoside were injected and the total quantity of ASFS excreted in the urine for the twenty four hours following injection was 33 mg. The normal excretion of ASFS for this person averaged 8 mg. per 24 hours. The amount of desoxycorticosterone glucoside which was recovered in the urine amounted, therefore, to 76% of the administered dose.

In this experiment the hot oxidation method was employed for estimation of ASFS.

e. Effect of administered ACTH on urinary excretion of ASFS

Figure 14

Urinary excretion of acid stable formaldehydogenic steroids (expressed as mg. desoxycorticosterone) by a normal male following injection of desoxycorticosterone glucoside.

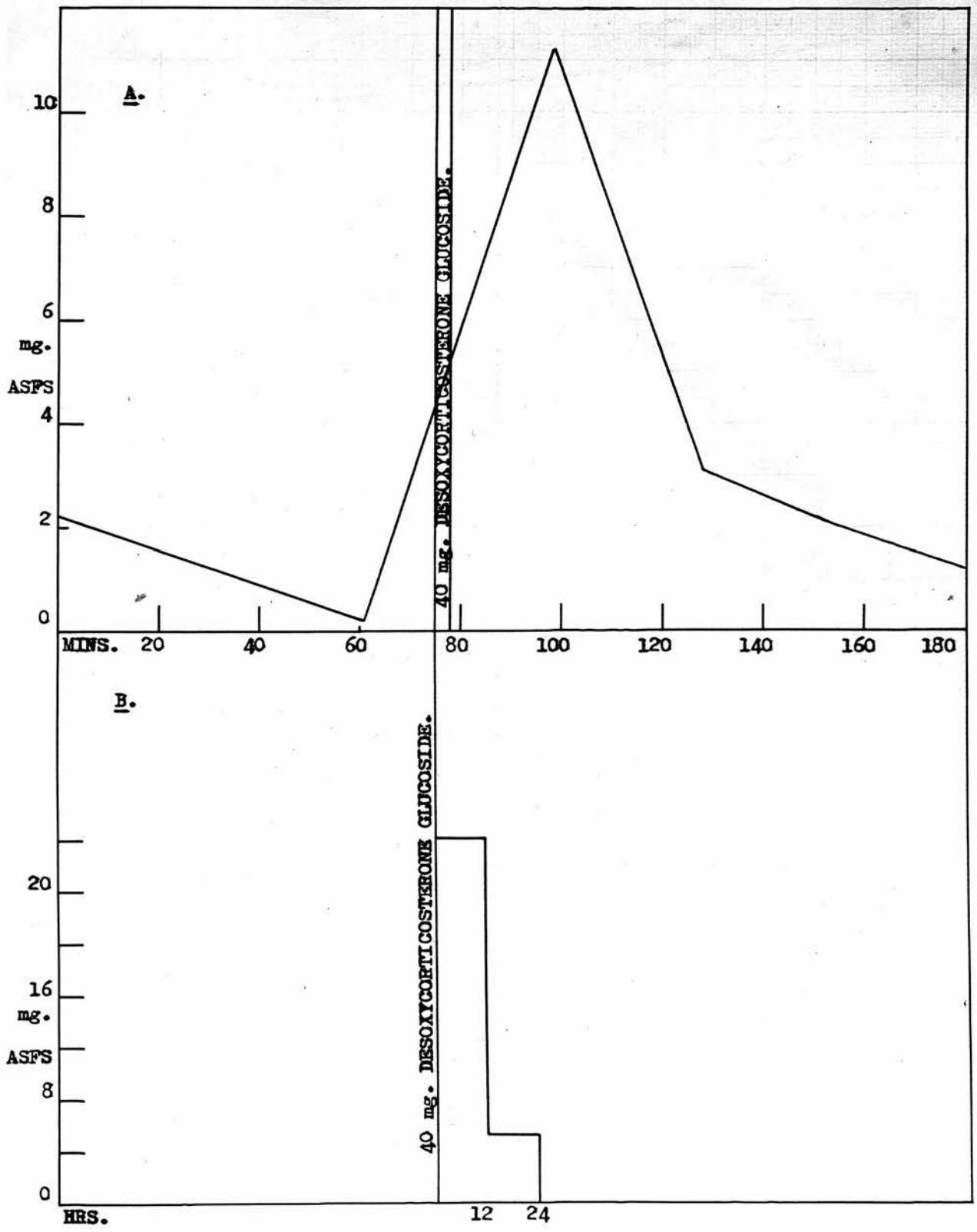


Figure 15 shows the effects of ACTH, administered to patients with various diseases, on urinary excretion of ASFS.

During the period when the patients were receiving ACTH it was observed that the quantities of ASFS excreted in twenty four hours were more variable from day to day than during the control period. In every case, however, a distinct rise in the corticosteroid fraction in the urine was evident immediately after administration had commenced and this increase persisted throughout the period when the hormone was being given.

The response to ACTH was evident whether the hot or cold oxidation method was used for the estimation of ASFS.

f. Effect of administered ACTH on urinary excretion of 17 ketosteroids

Figure 16 shows the effects of ACTH, administered to two normal females and one female patient with idiopathic hirsutism on urinary excretion of 17 ketosteroids.

Each of these subjects received 25 International Units ACTH per day for two days. In the first two cases the hormone was administered in a single intramuscular injection and in the third case intravenously by drip over 8 hours each day.

Figure 15

Effect of ACTH on urinary excretion of acid stable formaldehydogenic steroids (expressed as mg. desoxycorticosterone)

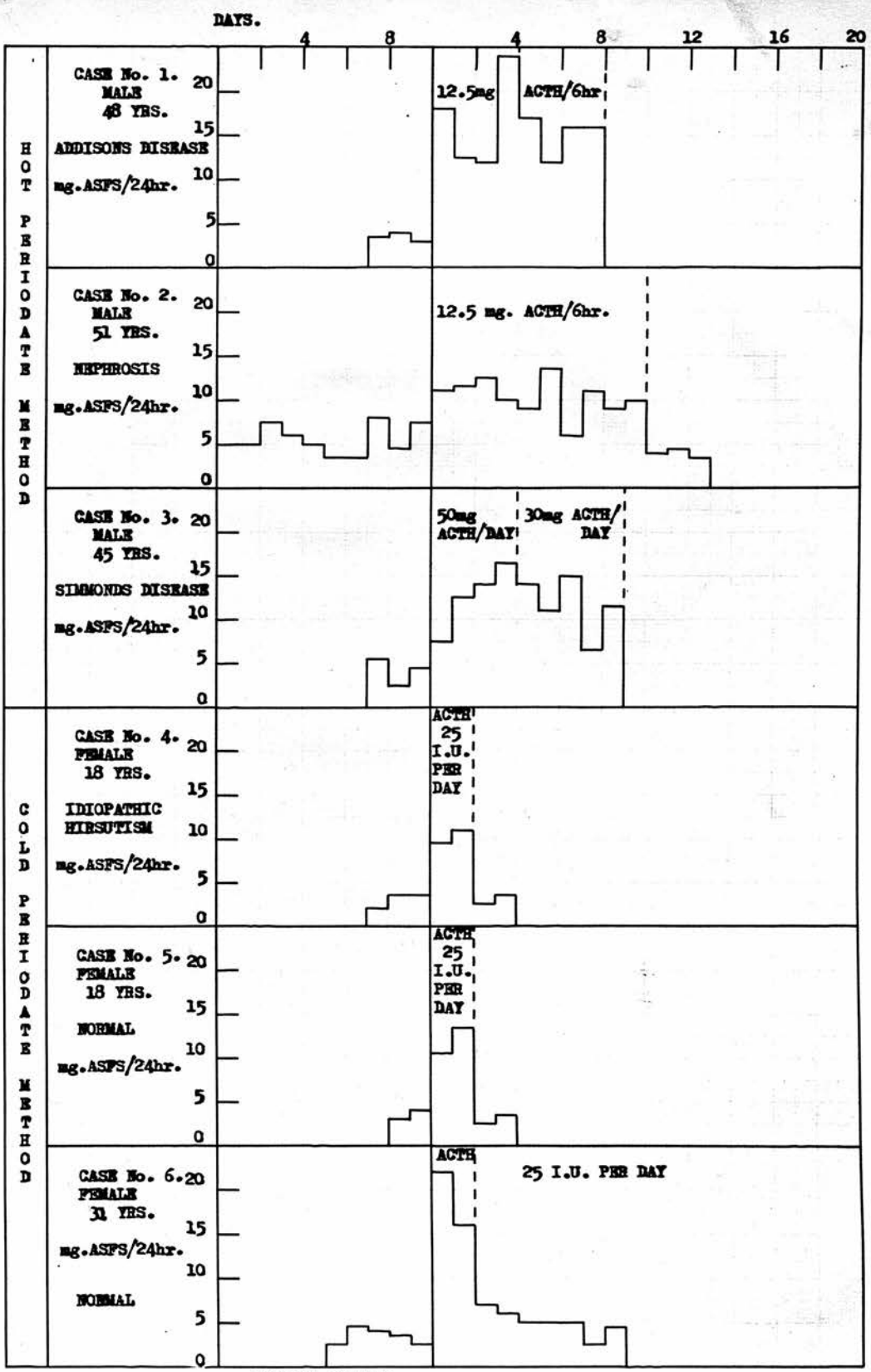
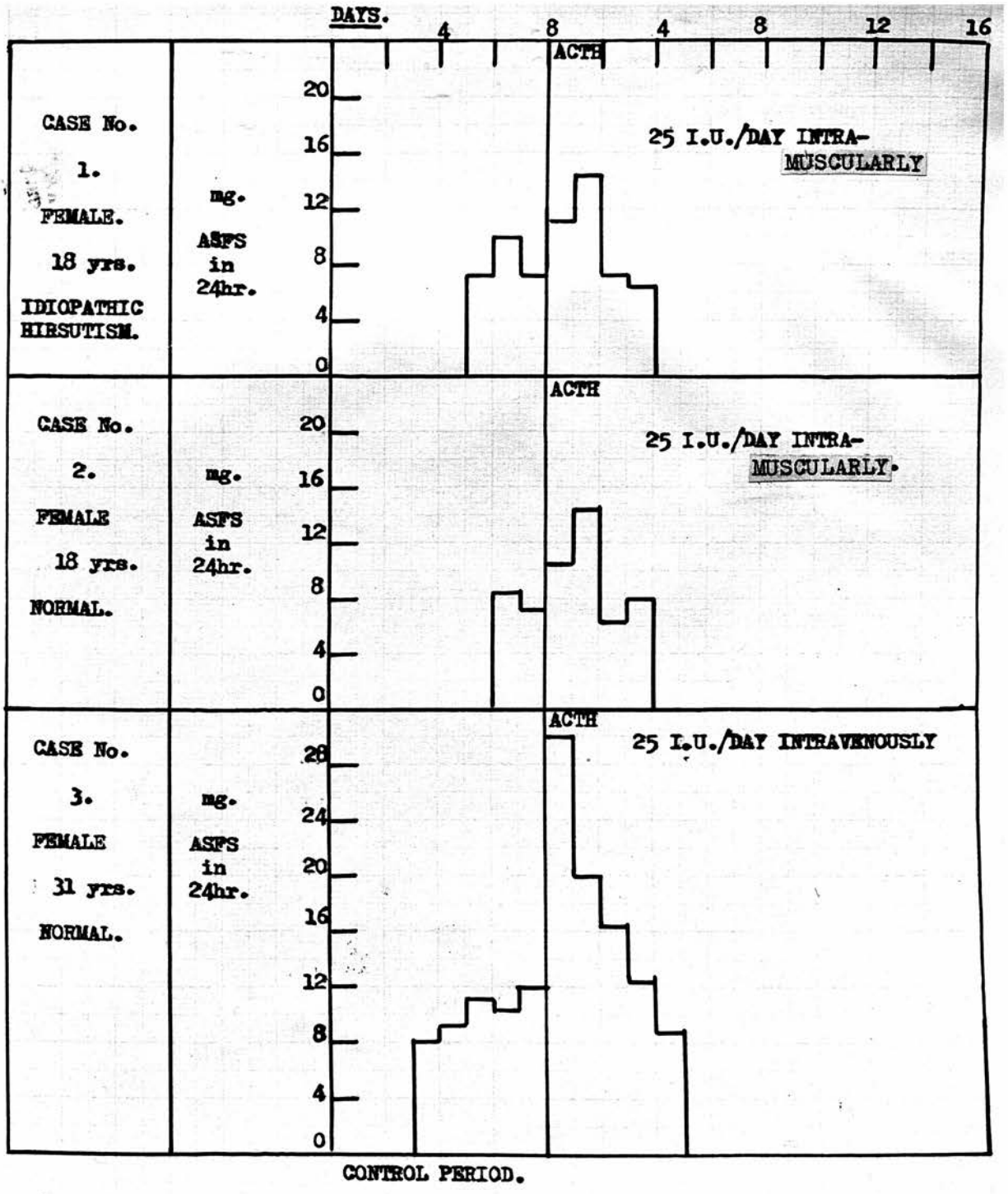


Figure 16

Effect of ACTH on urinary excretion of 17 ketosteroids.



All three patients showed a distinct increase in the amounts of 17-ketosteroids excreted after ACTH had been given. In the first two cases the maximum excretion was observed on the second day of hormone administration but in case no.3 this occurred in the first twenty four hours. The magnitude of the response was greatest in the third case.

6. DISCUSSION

a. Hot and cold methods for estimation of ASFS in urine

In the experiments which were first carried out on the estimation of acid stable formaldehydogenic steroids the hot method of oxidation was employed. This gave higher results than the cold oxidation procedure so that some other substance (or substances) which was oxidised by periodic acid at boiling point but not at room temperature must have been included in the estimation.

Recovery figures for pure steroids added to water and urine were similar for both methods and each procedure afforded some measure of adrenal activity since a rise in the excreted ASFS was invariably observed after administration of adrenocorticotropic hormone to experimental subjects. For any one person the concentration of ASFS in the urine remained

reasonably constant from day to day regardless of the temperature of oxidation employed in the method of estimating this corticosteroid fraction.

It seems reasonable to conclude, therefore, that if a change in adrenal cortical function brought about increased or decreased excretion of ASFS this would be evident whether measurements were made using hot or cold oxidation methods.

b. Recovery of steroids added to water and urine and administered to patients

When desoxycorticosterone was added to water its recovery was complete. From urine it was recovered to the extent of approximately 90% as was its acetate. This finding confirms the fact that acid stable formaldehydogenic steroids include compounds with a side chain structure similar to corticosterone and desoxycorticosterone.

Recovery figures for cortisone added to water or urine varied between 35% and 63% and indicated that any estimation of ASFS would include a certain proportion of compounds with a side chain structure similar to cortisone. The results of the experiments in which cortisone was administered to patients showed, however, that such compounds were not likely to be

included in an estimation of ASFS in urine. In these cases no increase or, at most, an increase equal to less than 10% of the administered dose was observed. In contrast administration of desoxycorticosterone glucoside caused an immediate and striking rise in urinary excretion of the corticosteroid fraction estimated by this procedure.

The method has been criticised by Marrian and Atherden (1953). These workers found, in agreement with the results reported here, that the formaldehydogenic properties of cortisone were not completely destroyed when the compound was added to water or urine and submitted to the acid hydrolysis process. They further observed an increased urinary excretion of ASFS after cortisone had been administered in a case of rheumatoid arthritis. On the basis of these results they suggested that ASFS in urinary extracts might largely consist of artefacts from C_{21}17OH formaldehydogenic steroids originally present in the urine.

In presenting the results of the experiments here it has been pointed out that cortisone did, in some cases, bring about an increase in urinary levels of ASFS but that this increase, in accordance with the findings of Marrian and Atherden, represented only a

small fraction of the administered steroid. Since cortisone added to water and urine may be recovered to the extent of 60% it is, of course, possible that acid stable formaldehydogenic steroids in urine may include a certain proportion of artefacts derived from C_{21}17OH compounds. In view of the low percentage recovery of cortisone in the urine of patients to whom it has been administered it seems reasonable to conclude that this will be a very small proportion.

The findings reported here which are the results of experiments carried out in collaboration with Dr.Tompsett do not entirely confirm the author's original claim that the method he described afforded a means of specifically estimating the metabolic products of hormones with a side chain structure similar to corticosterone and desoxycorticosterone. They do, however, indicate that the metabolic products of such compounds are largely of the type included in the acid stable formaldehydogenic steroid group and that cortisone and its excretory products are not predominantly of this type. Further any significant increase in urinary excretion of ASFS would more probably be a reflection of increased secretion of compounds resembling

corticosterone and desoxycorticosterone than of those resembling cortisone.

c. ASFS and 17-ketosteroids as indices of adrenal activity during salt deprivation

Corticosterone, desoxycorticosterone and other 17-hydroxy compounds are of the acid stable formaldehydogenic type. Although corticosterone is mainly glucogenetic in its activity, desoxycorticosterone is known to be potent in its effects on sodium metabolism. Moreover in circumstances where electrolyte metabolism is upset an increased urinary excretion of ASFS has been observed. (Wilkinson et al 1953)

Thus, without considering the method described by Tompsett as providing a means of assaying a specific salt and water hormone, it seemed possible that if altered adrenal function was concerned in the conservation of sodium observed when salt was removed from the food of the hypertensive and normotensive subjects in the experiments previously described, this changed activity might be reflected in altered excretion of ASFS. It was further considered that examination of 17-ketosteroid excretion under such conditions would be of interest. Although, in the male, these compounds may be derived from testicular steroids they are

largely the metabolic products of adrenal cortical hormones.

7. CONCLUSIONS

1. The amounts of ASFS and 17-ketosteroids in urine may reflect variations in adrenal activity as was shown by the increased excretion of these compounds which occurred when excessive adrenal activity was stimulated by administration of ACTH.

2. The metabolic products of cortisone are largely such as will escape detection in an estimation of ASFS.

3. The metabolic products of desoxycorticosterone excreted in the urine are predominatly of the type which will be included in an estimation of ASFS.

4. It is possible that a substantial increase in ASFS excretion may indicate an increase in the secretion of that group of steroids which includes the hormone or hormones especially concerned with water and electrolyte metabolism.

SECTION II - STEROIDS

PART III - SODIUM CONSERVATION IN HYPERTENSIVE AND NORMOTENSIVE SUBJECTS ON A SALT FREE DIET

1. INTRODUCTION

The experimental work which will be reported in the following pages was concerned with the amounts of certain steroid metabolites excreted daily in the urine of hypertensive and normotensive subjects during a control period and after severe restriction of dietary salt. It was hoped that changes in adrenal activity might be reflected in increased or decreased levels of the urinary corticoids under study so that information might be gained on two problems.

(a) Adrenal cortical activity in hypertensive as compared with normotensive subjects.

(b) The possible role of the adrenal cortex in the conservation of the body's supplies of salt when this commodity is removed from the food.

Before reporting the results of these investigations it seems relevant to consider the evidence which indicates (a) that the adrenal cortex is related in some fashion to the hypertensive state and (b) that the gland is concerned in the regulation of renal salt excretion.

a. The adrenal cortex and hypertension

In adrenal insufficiency low blood pressure is a frequent finding, and conversely, in cases of hyperadrenalism, hypertension may be evident. These facts indicate that the adrenal cortex is concerned in the maintenance of blood pressure at normal levels and appear to relate the glands to the hypertensive state. There is, however, no evidence to prove that adrenal cortical activity provides a primary cause of hypertension and, indeed, certain forms of the disease may exist in the absence of intact adrenals. (Perera 1951)

In 1939 Loeb and his associates first noted a rise in the blood pressure of two patients with Addisons disease, treated with DCA and, since then, the effects of adrenal cortical preparations on blood pressure have been the subject of frequent study. The pressor effects of many different steroid compounds have been demonstrated and it has also been shown that administration of large doses of ACTH to patients may result in elevation of blood pressure. (Swingle and Remington 1944, Derbes and Weiss 1951)

A further rise in blood pressure in cases of hypertension on administration of DCA together with salt was noted by Perera and Blood

(1947). It is of interest that the pressor effect of the steroid compound was only evident if salt was given simultaneously. In view of this result it seems possible that, the action which causes lowering of blood pressure when salt restriction is used as a therapy in the treatment of hypertension, is mediated through an adrenal mechanism. Although previous investigators were able to demonstrate only very minor changes in the blood pressure of normal healthy subjects on prolonged treatment with DCA and salt, Luft and Sjögren (1949). found that a small but definite increase could be produced in such people after about a week.

In summary, there is evidence to suggest that hypertension may be accompanied by some abnormality in adrenal function and, further, that the adrenal glands are concerned in the depressor effect which dietary salt restriction may have in the disease. It was considered, therefore, that daily measurement of certain steroid metabolites in the urine of the experimental subjects both before and after removal of dietary salt might reveal a difference between the two groups.

b. <u>The adrenal cortex and renal salt excretion</u>

In acute adrenal insufficiency a severe loss of body supplies of salt in the urine is

evident. Even when the concentration of sodium in the plasma is low increased excretion occurs. Under normal conditions a low plasma sodium level would lead to decreased urinary output and it follows that the action of the renal tubules in reabsorbing sodium must be depressed.

Logically one would expect that excessive activity of the adrenal glands would result in increased tubular reabsorption of sodium and that the high urinary output of Addisons disease would find its counterpart in a low excretion of sodium in a condition such as Cushings syndrome. In fact this has not been clearly demonstrated to be the case and there is experimental evidence which indicates that tubular reabsorption of the ion is not greater than normal in the latter condition. (Black 1952) Conn (1949), however, has found that the salt content of the sweat which is high in Addisons disease is low in Cushings syndrome which certainly suggests increased action of a salt retaining hormone and it may be that there is another factor to be considered which masks the adrenal action in reducing urinary excretion of sodium. Black (1952) suggests that the increased excretion of potassium which is to be observed in Cushings syndrome may be

responsible, since potassium diuresis is known to increase the rate of urinary output of sodium. Excessive activity of the adrenal gland induced by administration of ACTH has frequently resulted in sodium retention.

In summary, when adrenal hormones are deficient or lacking tubular reabsorption of sodium is depressed. It is probable that hyperactivity of the adrenal cortex is associated with increased production of a salt retaining hormone although this has not been conclusively proved to be that case. It seems probable, therefore, that the prolonged increase in sodium reabsorption observed in patients on a low salt diet is due to increased adrenal cortical action. This possibility might be strengthened if it could be shown that an adrenal cortical hormone (or hormones) could produce decreased salt excretion and, at the same time, changes in the urinary levels of other metabolites parallel to those observed under low salt therapy.

It has previously been reported that the hypertensive and normal subjects under study responded to withdrawal of salt from the diet by reducing the amounts of sodium and chloride in the urine and at the same time, increasing the quantity of potassium to the extent that a

temporary negative balance was created for this ion. Increased excretion of potassium was not due to catabolism of protein since only very minor changes in nitrogen excretion occurred. The effects of administration of individual hormones of the adrenal cortex on urinary excretion of sodium, potassium, chloride and nitrogen in experimental subjects and in patients will now be considered.

2. EFFECTS OF ADMINISTRATION OF ADRENAL CORTICAL HORMONES

Adrenal cortical hormones may affect the following processes:

1. Regulation of electrolyte metabolism.
2. Regulation of carbohydrate, fat and protein metabolism.
3. Regulation of lymphoid tissue and circulating eosinophils.
4. Androgenic function.
5. Regulation of pigmentation.

It has not been proved that all these metabolic systems are not under the control of one adrenal hormone but since each compound which has been isolated from the adrenal cortex exerts its major functional effect in one or two such processes and appears to be only mildly effective in the other systems it seems reasonable to assume that the gland, under normal circumstances, secretes more than one type of

steroid.

This conception may prove to be wrong. Aldosterone which was recently isolated by Tait and his associates (1952) has proved to be exceedingly active in causing retention of sodium in adrenalectomised animals and in patients with Addisons disease. It is also potent in maintaining life in adrenalectomised animals (British Medical Journal 1954). Further study may conceivably demonstrate its efficacy in the other metabolic systems listed above but, at the present time, the compounds which are known to affect these processes most profoundly are as follows:

1. Electrolyte metabolism - Aldosterone and desoxycorticosterone.
2. Carbohydrate, fat and protein metabolism - 11; 17-oxygenated steroids (cortisone and 17-hydroxy corticosterone) and, to a lesser extent 11-oxygenated steroids (corticosterone and 11-dehydrocorticosterone).
3. Lymphoid tissue and circulating eosinophils - 11; 17-oxysteroids.
4. Androgenic function - Compounds related to androsterone. (Thorn and Forsham 1949)

In considering the effects of administration of cortical hormones on the metabolism of sodium, potassium and chloride it is impossible to disregard those steroids whose major functional effects are not

on electrolyte metabolism. The reports in the literature provide ample evidence of the fact that all the known adrenal steroids may, under particular conditions, stimulate the kidney tubules to increased reabsorption of sodium from tubular urine. When this occurs it is almost invariably accompanied by increased excretion of potassium. Further the 11, 17-oxy steroids and, to a lesser extent, the 11 oxy compounds can affect potassium metabolism by causing disintegration of certain tissues and consequent release of potassium to the extracellular fluids. In addition their effects on carbohydrate metabolism are associated with potassium when glycogen is stored.

It is, therefore, necessary when increased reabsorption of sodium is observed and the possibility of adrenal cortical action is being considered to study the probability that any (or more than) one of the known cortical hormones might have been concerned in producing such an increase. Finally, to survey the situation completely the effects of adrenocorticotropic hormone on electrolyte excretion cannot be overlooked since it is conceivable that the hormones of the adrenal cortex are secreted in response to stimulation by this agent.

a. Desoxycorticosterone

Sodium and Chloride

Until the discovery of aldosterone,

desoxycorticosterone was the most potent compound known in causing sodium retention in man and animals.

Reports in the literature on the effects of administration of desoxycorticosterone, its acetate or glucoside show that these almost invariably include reduced excretion of the sodium and chloride ions. This is true for normal subjects and for patients suffering from a variety of diseases, the apparent exception being Cushings syndrome. In this condition desoxycorticosterone causes increased salt excretion.

The power of the compound in causing sodium retention is such that it may be used as a replacement therapy in Addisons disease. It has also been found to reduce the salt concentration of sweat in both normals and Addisonian patients.

Potassium

Administration of desoxycorticosterone or its conjugates generally leads to increased excretion of potassium. It has been demonstrated in animals that overdosage may lead to depletion of body potassium to such an extent that paralysis or cardiac failure results.

Nitrogen

In the doses normally used therapeutically

desoxycorticosterone does not generally affect nitrogen balance. With larger amounts some investigators have noted a slight increase in nitrogen excretion.

(References: Barnet and Macnamarra 1949, Conn 1949, Daughaday and MacBryde 1950, Ferrebee et al 1939, Forsham 1950, Fourman et al 1952, Fourman et al 1949, Gaunt 1951, Hartman and Brownell 1949, Kendall 1948, Kriss and Futcher 1949, Luft and Sjögren 1952, MacGavack et al 1946, Soffer et al 1944, Thorn and Emerson 1940, Thorn and Forsham 1949.)

b. <u>11-desoxy-17-hydroxy corticosterone</u>

The few reports available in the literature on the effects of administration of this compound show it to have slight or no effect on salt excretion and no effect on either nitrogen or potassium balance.

(References: Fajans et al 1951, Pearson et al 1951)

c. <u>11 oxygenated steroids</u>

<u>Corticosterone and 11-dehydrocorticosterone</u>

<u>Sodium and Chloride</u>

Both these compounds appear to cause slight sodium retention in normals and to have a limited power to bring about increased reabsorption of the sodium and chloride ions in

Addisons disease.

Potassium

In general when the effects of administered 11-oxy-steroids on electrolytes have been studied increased excretion of potassium has been observed.

Nitrogen

Corticosterone and 11-dehydro-corticosterone administered to man do not appear to affect nitrogen excretion to any great extent although a slight increased excretion has been noted for both.

(Conn et al 1951 A & B, Homburger et al 1948, Sprague et al 1948, Thorn and Forsham 1949)

d. 11; 17-oxysteroids

Cortisone and 17-hydroxycorticosterone

Sodium and Chloride

The view of early investigators was that the characteristic effect of these steroids was to cause increased excretion of sodium and chloride in animals. The experiments which led to these conclusions were, however, limited by the fact that only small amounts of the steroids were then available and observations were of necessity confined to the effects observed after single doses of cortisone and 17-hydroxycorticosterone. This evidence led to the suggestion that an important function of the

11;17-oxy steroids in electrolyte metabolism was to counterbalance the salt retaining activity of compounds like desoxycorticosterone, and that the maintenance of salt equilibrium depended upon balance between the two types of steroid. Later workers however demonstrated that the increased salt excretion in animals was only temporary, lasting from 1 - 3 days and that normal balance was rapidly restored.

The reports in the literature to-day on the effects of administration of this group of steroids are confusingly variable. It appears that the changes observed in electrolyte excretion depend on (a) dose, (b) duration of administration and (c) functional state of the adrenal cortices of the recipient.

It is generally apparent that cortisone and 17-hydroxy-corticosterone are less potent in their effects on electrolyte metabolism than is desoxycorticosterone. Cortisone has been reported to cause sodium retention in normal people and during the course of Addisons disease, hypertension, rheumatoid arthritis and other abnormal conditions. Frequently this retention has been only transient. Increased excretion of salt has also been reported in normal and diseased conditions, generally when the period of administration has been curtailed and, to

add to the complexity of the situation, investigators have reported initial excretion followed by retention and initial retention followed by excretion.

It is evident that cortisone and 17-hydroxy -corticosterone are variable in their effects on salt metabolism and may produce excretion or retention of sodium and chloride depending entirely upon the conditions.

Potassium

The compounds have been observed to cause increased excretion of potassium in a variety of clinical and experimental conditions. The increased amounts of potassium in the urine may be derived from both intracellular and extracellular fluids, the portion from the extracellular fluid compartment being, in all probability, a manifestation of protein break down.

Nitrogen

Early experiments on animals established the fact that the compounds are capable of inducing either failure of anabolism or catabolism of protein, and it is evident from the literature that administration of cortisone and 17-hydroxy corticosterone to patients suffering from various diseases and to normal persons may give rise to increased urinary

excretion of nitrogen.

In general, only slight changes in nitrogen balance have been noted when the dose of cortisone has been 100 mg. per day or less. Doses of more than 100 mg. per day have resulted in marked changes in nitrogen excretion and negative nitrogen balance.

(References: Conn et al 1951,
Forsham et al 1949, Forsham 1950, Gaunt 1951,
Hartman and Brownell 1949, Ingle and Baker 1953,
Kendall 1948, Luft and Sjögren 1951,
Perera et al 1949, Sprague et al 1948, 1950, 1951,
Sprague 1951, Thorn et al 1951,
Thorn and Forsham 1949, Woodbury et al 1950.)

e. ACTH

Sodium and Chloride

Similarly to the 11, 17-oxy steroids ACTH appears to be capable of producing salt retention or salt excretion in man. It has been reported in the literature to cause both these effects but generally sodium phoresis is only to be observed in short term experiments or curtailed periods of administration. Salt retention produced by ACTH administration appears, in general, to be of short duration.

Potassium

During administration of ACTH to normal people and to patients suffering from various

diseases increased excretion of potassium has been observed. Again the potassium may be derived from both intra-and extracellular fluids, the latter being a manifestation of protein breakdown.

Nitrogen

Like the 11; 17-oxy steroids ACTH may produce marked changes in nitrogen balance on administration to man. Generally the dose of ACTH required to produce these changes is lower than that of cortisone and negative nitrogen balance follows daily administration of 100 mg. ACTH.

It is evident that the effects of ACTH on the metabolism of sodium, potassium, chloride and nitrogen are very similar to those of cortisone and 17-hydroxy corticosterone, probably due to the fact that ACTH stimulates the secretion of these compounds by the adrenal glands.

(References: Conn et al 1951 A & B, Forsham et al 1948, Gaunt 1951, Hartman and Brownell 1949, Kinsell et al 1950, Luft and Sjögren 1949, MacIntosh et al 1950, Mason et al 1947, Renold et al 1952, Soffer 1950, Sprague 1951, Sprague et al 1951, Wilkins et al 1950.)

f. Summary

While all the adrenal cortical hormones

just considered, with the possible exception of 11-desoxy-17-hydroxy corticosterone may, on administration, bring about changes in electrolyte excretion it is evident that all are not equally potent in this respect. It appears that those compounds which are most potent in their effects on carbohydrate metabolism are most variable in the effects which they produce in electrolyte excretion. Table 14 illustrates this point, for those hormones which have been most studied (excluding aldosterone).

Despite the fact, however, that all adrenal cortical hormones do not influence electrolyte balance to the same degree it is clear that any one of the steroids listed above might, on administration, produce salt retention and potassium excretion without any accompanying changes in nitrogen balance. It is more probable that such effects would be observed after DCA had been given to a patient or experimental subject. It is unlikely that administered ACTH would give rise to the alterations in electrolyte excretion without simultaneous increase in urinary nitrogen.

The effects of adrenal cortical hormones, ACTH and a low salt diet on excretion of sodium, potassium, chloride and nitrogen in the urine are summarised in Table 15.

Table 14

Carbohydrate and electrolyte regulating effects of adrenal cortical steroids (Thorn and Forsham 1949).

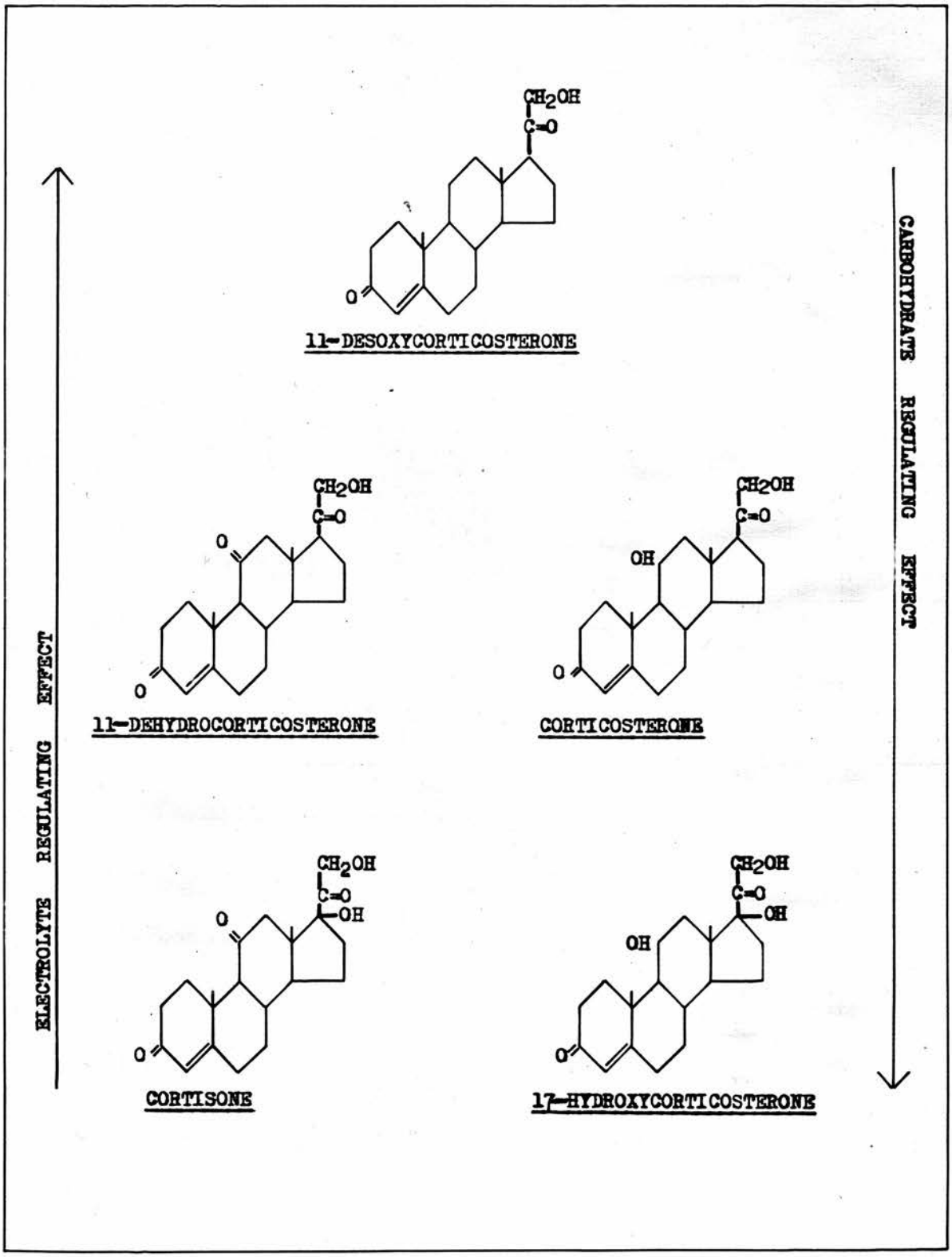


TABLE 15

The effects of adrenal cortical hormones, ACTH and a low salt diet on the excretion in the urine of sodium, potassium, chloride and nitrogen

Compound	Na	K	Cl	N
Aldosterone	decreased excretion	increased excretion	decreased excretion	?
Desoxycorticosterone	decreased excretion except in Cushings syndrome	increased excretion	decreased excretion except in Cushings syndrome	slight or no change
11-desoxy-17-hydroxy corticosterone	slight or no change	no change	slight or no change	no change
11-oxy steroids	slightly decreased excretion	slightly increased excretion	slightly decreased excretion	slight or no change
11-17-oxy steroids	variable depending on conditions	increased excretion	variable depending on conditions	slightly o markedly increased excretion
ACTH	variable depending on conditions	increased excretion	variable depending on conditions	increased excretion
Low salt diet	decreased excretion	temporary increased excretion	decreased excretion	slight or no change

In conclusion, the theory that the adrenal cortex is concerned in the mechanism which ensures protection of the essential salt supplies of the body when sodium chloride is no longer being ingested receives some support from the fact that adrenal cortical hormones might, on administration produce changes in electrolyte balance similar to those observed in experimental subjects on a low salt diet.

Thus it was considered that study of the daily amounts of certain steroid metabolites in the urine of these subjects would be of considerable interest and might, by revealing a change in levels after restriction of dietary salt, provide some index of altered adrenal activity.

Although the method used for estimation of corticoids in urine probably measures only a small fraction of total adrenal output it has proved to be useful in measuring adrenal activity and was therefore considered to be suitable for the purpose.

3. PROCEDURE

For the normotensive and hypertensive subjects on a salt free diet the procedure was as reported in Section I.

In addition estimations of ASFS were carried out on the urine of several hypertensive patients and

normal persons for the purpose of comparing the amounts excreted by those subjects exhibiting high blood pressure with the amounts excreted under normal conditions.

4. METHODS

(a) Acid stable formaldehydogenic steroids. As described.

(b) 17-ketosteroids. As described.

(c) 17-ketogenic steroids.

17-ketogenic steroids were estimated daily in the urine of two hypertensive patients during a control period and after removal of salt from the diet. The method used was a modification of that proposed by Norymberski and Stubbs (1952). After oxidation of the urine specimen with sodium bismuthate a second estimation of 17-ketosteroids by the method already described gave a value for total 17-ketosteroids.

Total 17-ketosteroids - 17-ketosteroids = 17-ketogenic steroids.

The oxidation with sodium bismuthate was carried out as detailed below.

Reagents: Acetic acid. A.R. Glacial.

Sodium Bismuthate A.R.

5% W/V sodium metabisulphite.

Method. Into a 100 ml. flask fitted with a ground glass stopper were measured 8g. sodium bismuthate, 20 ml. urine and 20 ml. glacial acetic acid. The

flask was stoppered and shaken in the dark for 30 minutes and then the contents were transferred to a tube and centrifuged for 10 minutes. The supernatant fluid was decanted. The precipitate was washed with 20 ml. water, the water washings added to the supernatant fluid and an estimation of 17-ketosteroids was carried out on the combined collections.

5. RESULTS

a. Excretion of ASFS in urine by hypertensive and normal subjects

Table 16 shows the average amount of acid stable formaldehydogenic steroids excreted daily in the urine of (a) six hypertensive and seven normotensive subjects as estimated by the hot periodate method of oxidation, and (b) four hypertensive and nine normotensive subjects as estimated by the cold periodate method of oxidation.

It was found that the mean value for the hypertensive group was slightly higher than the mean value for the normotensive group, irrespect-ive of the temperature of oxidation. The significance of this finding was evaluated from the formula $t = \frac{m_2 - m_1}{\sqrt{SE_2 + SE_1}}$ where m represents mean and SE represents standard error, and t was checked against Fisher's tables. The difference between the two groups of experimental subjects

Table 16

Excretion of Acid Stable Formaldehydogenic Steroids in the Urine of Hypertensive and Normotensive Subjects

	Normotensive Subjects					Hypertensive Subjects				
	Subject No.	Sex	Age	No. of consecutive days on which ASFS estimated	Average daily excr. mg/24 hr.	Subject No.	Sex	Age	No. of consecutive days on which ASFS estimated	Average daily excr. mg/24 hr.
Hot Oxidation Method	1	F	19	2	5.6	1	F	51	2	5.6
	2	F	35	1	6.2	2	F	47	2	8.6
	3	F	42	1	4.9	3	F	66	1	4.7
	4	F	25	3	7.2	4	F	43	2	4.9
	5	F	26	1	5.3	5	F	43	1	15.0
	6	F	21	1	5.5	6	F	46	1	7.1
	7	F	43	6	10.2					
Cold Oxidation Method	1	F	34	6	2.3	1	F	40	6	1.6
	2	M	54	6	4.1	2	M	46	6	3.5
	3	M	53	6	1.8	3	F	48	6	8.4
	4	M	21	6	3.7	4	M	42	4	6.4
	5	M.	36	6	4.1					
	6	M	26	1	4.2					
	7	M	26	1	3.9					
	8	F	47	6	3.8					
	9	M	54	4	3.0					

Method	Mean Value for Normotensive Subjects	Mean Value for Hypertensive Subjects	Probability of difference between Hypertensives and Normals being due to chance (ρ)
Hot oxidation	6.4±1.8	7.6±3.8	0.5
Cold oxidation	3.3±0.2	4.9±3.0	0.4

To be significant P must be < 0.05

Table 17

Excretion of 17-ketosteroids in the urine of hypertensive and normal subjects.

Normotensive Subjects					Hypertensive Subjects				
Subject No.	Sex	Age	No. of consecutive days on which 17 KS estimated	Average daily excretion mg/24 hrs.	Subject No.	Sex	Age	No. of consecutive days on which 17 KS estimated	Average daily excretion mg/24 hr
1	M	53	6	13.6	1	F	40	6	3.7
2	M	54	6	10.5	2	M	46	6	8.1
3	M	21	6	12.8	3	F	48	8	4.9
4	M	36	6	18.5	4	F	39	6	13.3
5	M	45	2	18.0					
6	F	47	6	3.8					
7	M	34	4	9.7					
			Mean = 12.4 ± 5.1					Mean = 9.5 ± 3.4	

P (probability that difference between two groups is due to chance) = 0.3

To be significant P must be < 0.05

was found to be insignificant.

This does not, of course, prove that the excretion of ASFS in cases of hypertension does not vary from normal. The total number of experimental subjects was too small to allow definite conclusions to be drawn from the results of the estimation and, further, the individuals with normal blood pressure outnumber-ed those showing abnormal levels. The age range was not similar in each group and only two out of ten hypertensive patients were male while the normal cases included six males and ten females.

It is of interest however that the excretion of ASFS in the urine of the particular people under study revealed no significant difference between hypertensive and normotensive persons and provided no index of increased adrenal activity in the former group.

b. Excretion of 17-ketosteroids by hypertensive and normotensive subjects

Table 17 shows the amounts of 17-keto-steroids excreted daily in the urine of seven hypertensive and four normotensive subjects. The mean value for the former was found to be considerably lower than a similar value for the latter.

This difference proved to be insignificant

When evaluated by the formula quoted above. The comparison is not, in any case, entirely satisfactory since the sex of the normotensive subjects was predominantly male, and although 17-ketosteroids are formed primarily from the secretions of the adrenal glands they are also derived, in the male, from testicular steroids.

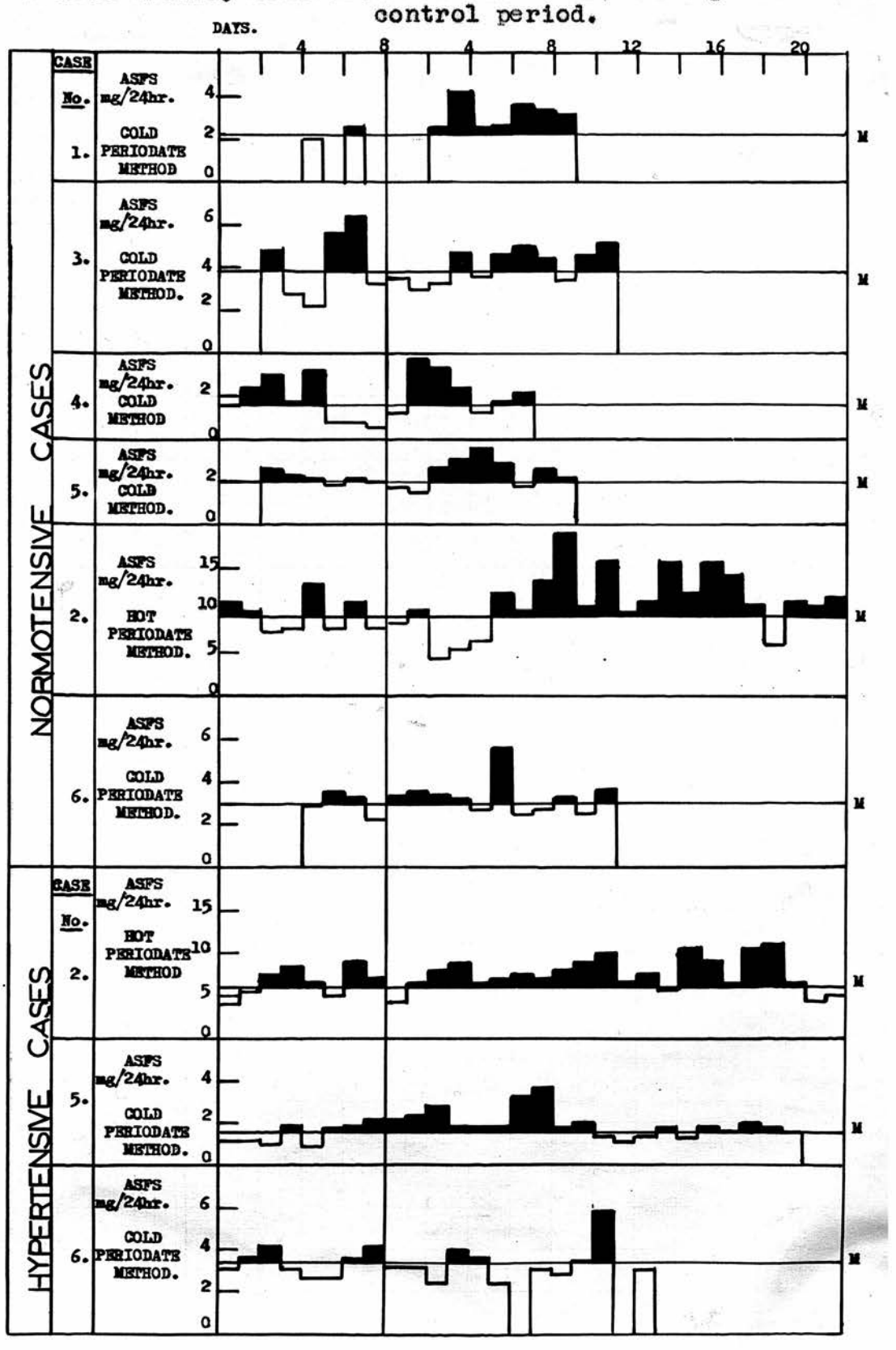
c. Urinary excretion of ASFS during a control period and after withdrawal of salt from the diet

Figure 17 shows the amounts of acid stable formaldehydogenic steroids excreted in the urine of six normotensive subjects and three hypertensive subjects during a preliminary control period and after removal of salt from the diet.

Three normal subjects (nos. 2, 4 and 5) showed a slight but definite increase in urinary steroid levels for a few days during the period of salt deprivation. Of the three remaining cases one (no.1) showed an apparent temporary increase in the amount of ASFS excreted daily when the ingestion of salt was arrested but, since it was only possible to carry out estimations on two twenty four hour urine collections during the control period, this increase could not be regarded as definite. Subject no.6 evidenced a high urinary excretion

Figure 17

Urinary excretion of acid stable formaldehydogenic steroids (expressed as mg. desoxycorticosterone) by hypertensive and normal subjects during a control period and after removal of salt from the diet. M=mean urinary excretion ASFS in 24 hr. during the control period.



of ASFS on one day during the time when salt was lacking in the diet. On that particular day, however, the urine volume also showed an increase so significance could not be attached to the increased amount of ASFS excreted.

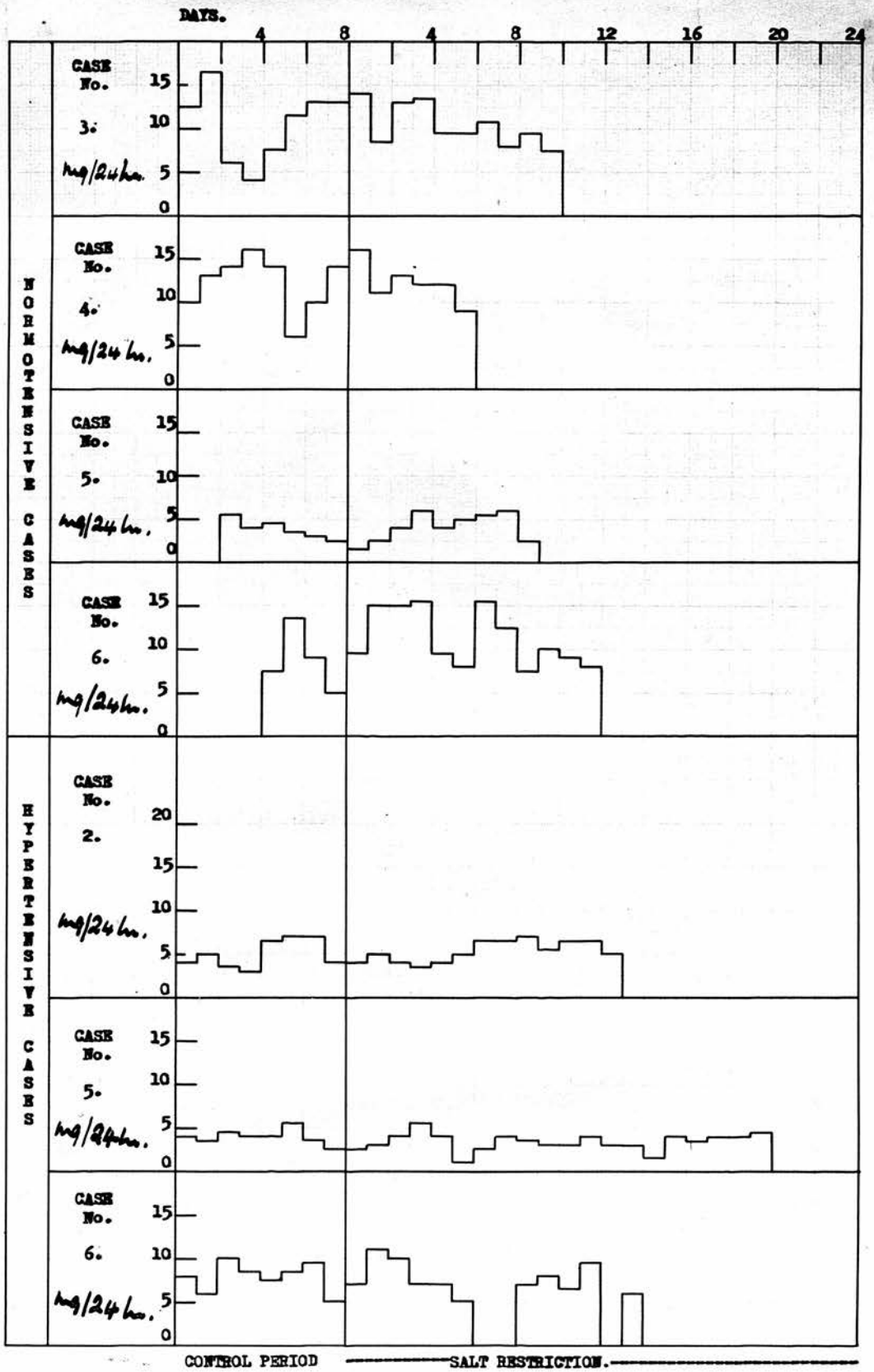
In the hypertensive group results were similar. Two subjects, (nos. 1 and 2) showed a slight but distinct increase in the amounts of ASFS present in the twenty four hour urine collections for a few days during the salt deprivation period. In case no.3 an elevated urinary output was evident on one day.

d. Urinary excretion of 17-ketosteroids during a control period and after removal of dietary salt

Figure 18 shows the amounts of 17-ketosteroids excreted in the urine of four normotensive subjects and three hypertensive subjects during a preliminary control period and after removal of salt from the diet. The urinary output of these steroids was rather variable during the control period in spite of the constancy of the daily diet and this variability continued after withdrawal of sodium chloride from the food. No consistent or definite difference could be observed between the amounts of 17-ketosteroids excreted in the urine during the control period and those

Figure 18

Urinary excretion of 17-ketosteroids by hypertensive and normal subjects during a control period and after removal of salt from the diet.



excreted at any time during the period of salt deprivation, either for hypertensive or for normal subjects.

e. Urinary excretion of 17-ketogenic steroids during a control period and after removal of salt from the diet

Figure 19 shows the amounts of 17-ketogenic steroids excreted daily in the urine of two hypertensive subjects, one male and one female. The urinary output of these steroids was found to be exceedingly variable from day to day and no difference could be observed between the amounts excreted during the control periods and the periods of salt restriction.

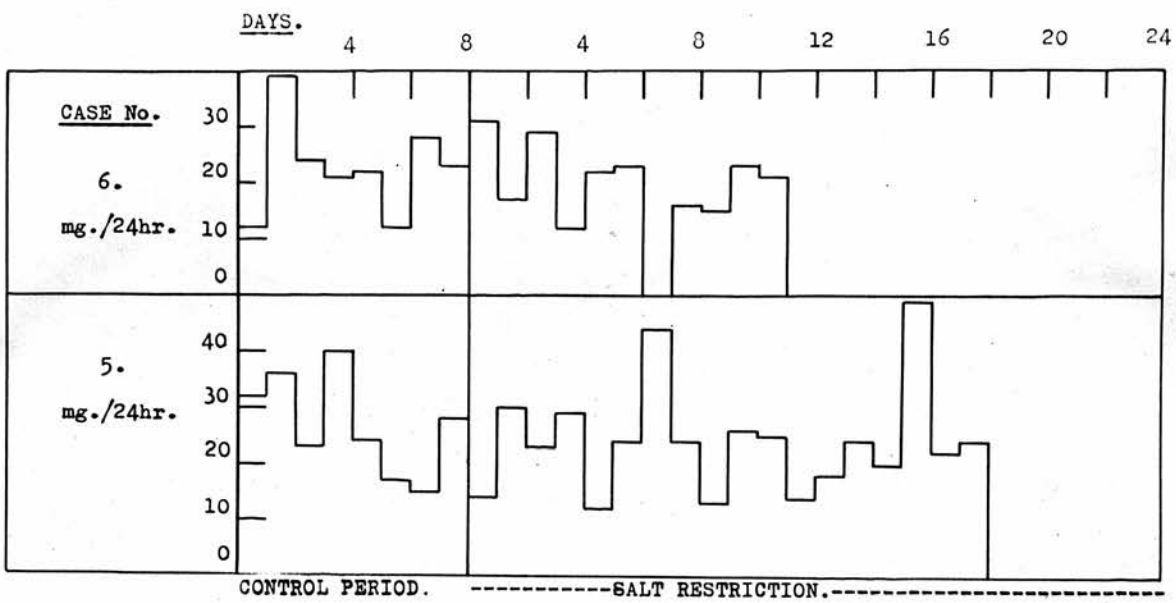
6. DISCUSSION

a. Excretion of acid stable formaldehydogenic steroids and 17-ketosteroids in the urine of hypertensive and normotensive individuals

Estimations of the amounts of ASFS excreted in the urine of ten hypertensive and sixteen normotensive subjects revealed no significant difference between mean values for the two groups. This is in accordance with the results reported by other investigators who have studied the urinary output of formaldehydogenic steroids in cases of hypertension. Daughaday et al (1949 B) reported that patients suffering from

Figure 19

Urinary excretion of 17-ketogenic steroids by two hypertensive subjects during a control period and after removal of salt from the diet.



the disease excreted normal amounts of free formaldehydogenic steroids and Tobian (1949) concluded that, in most cases of essential hypertension, the amounts of such compounds present in the urine were within the normal range. Since free formaldehydogenic steroids and ASFS most probably represent only a fraction of the total urinary corticosteroid content these findings do not, of course, preclude the possibility that urinary levels of other steroid metabolites vary from normal in the hypertensive condition.

In reporting a study of the excretion of 17-ketosteroids in the urine of 14 normal and 40 hypertensive females, Bruger et al (1944) concluded that the latter subjects excreted a significantly lower quantity than the former. They found that, out of the 14 normotensive persons studied, 4 showed levels of excretion within the range 2.5 - 12.5 mg. per twenty four hours and 10 were within the limits 12.5 - 22.5 mg. per twenty four hours. Dividing the hypertensive group in this way, 37 fell into the lower range and 3 into the higher.

It has already been pointed out that the comparison made between 17-ketosteroid excretion in the hypertensive and normotensive cases reported here was not entirely satisfactory

since the sex of the second group was predominantly male and the total number of experimental subjects was small. It is of interest, however, that these cases show five out of seven normotensive subjects who excreted amounts of 17-ketosteroids within the range 12.5 - 22.5 mg. per twenty four hours while only one of the four hypertensive subjects excreted an amount greater than 12.5 mg. per twenty four hours.

A low level of 17-ketosteroid excretion would not, in itself, afford evidence of reduced activity of the adrenal gland. This might be so, however, if it were shown that, coincident with the decreased excretion of 17-ketosteroids there was no increase in any fraction of the other corticosteroids present in urine.

b. Urinary excretion of 17-ketosteroids and 17-ketogenic steroids by hypertensive and normotensive subjects during a control period and after removal of salt from the diet

The fact that the amounts of 17-ketosteroids present in the daily collections of urine from the persons used for this study were rather variable during the control periods makes it difficult to place an interpretation upon the results. There was, however, no distinct increase or decrease in the levels of these metabolites excreted by three hypertensive and

four normotensive individuals after ingestion of salt had stopped. Thus, utilising the urinary output of 17-ketosteroids as an index of adrenal cortical activity no evidence was obtained of any alteration in such activity in response to the removal of salt from the food.

This is in accordance with the findings of Daughaday and MacBryde (1950) who concluded from their investigations on urinary steroid excretion during salt deprivation that a correlation did not exist between 17-ketosteroid output and sodium intake.

Similarly estimation of 17-ketogenic steroids in the urine of two hypertensive subjects afforded no indication of altered adrenal activity when salt ingestion had ceased. No definite difference could be discerned between the amounts excreted during the control period and those excreted during the period of salt deprivation.

c. Excretion of ASFS in the urine of hypertensive and normotensive subjects during a control period and after removal of salt from the diet

A slight but definite increase in the amounts of ASFS present in the urine of three out of the six normotensive subjects and two out of the three hypertensive patients who were used

in this study was observed during the time when salt was restricted in the food. Since all other constituents of the diet and all experimental conditions were constant throughout the period of study it is logical to conclude that this rise in urinary levels of ASFS was a result of sodium chloride deprivation.

Investigators who have studied the excretion of free formaldehydogenic steroids in urine after withdrawal of salt from the diet have observed no increase in the amounts of these compounds excreted by normal persons and by patients suffering from various diseases. Daughaday and MacBryde (1950) reached the conclusion that, despite the remarkable sodium conservation to be observed when experimental subjects underwent sodium deprivation, no increased adrenal activity was evident when measurement of free formaldehydogenic steroids was used as the index of adrenal function. Lloyd (1952) and his associates noted no increase in the levels of such compounds after sodium chloride was removed from the diet even when a potassium cation exchange resin was administered to reduce sodium intake as far as possible.

In view of these findings it seems possible that the acid stable group of formaldehydogenic steroids in urine may include a sodium retaining factor which is not estimated with the free

formaldehydogenic steroids.

Recently Genest (1954) reported the results of an extensive study on steroid excretion by hypertensive patients under low salt therapy. This investigator restricted the sodium intake of seven patients with essential hypertension for prolonged periods. He found that the state of adaptation of the seven patients in response to a severe restriction of salt in the food, whether or not there was any change in blood pressure was not accompanied by any significant modification in seven urinary steroid fractions estimated after separation by chromatographic fractionation procedure.

To reconcile this finding with the results reported here is difficult. One explanation does, however, present itself. Genest found that administration of desoxycorticosterone acetate to one patient with essential hypertension did not result in any significant difference in the appropriate steroid fraction in the urine from control level. The author concluded that desoxycorticosterone acetate is metabolised in the body or excreted in a way which made it impossible to determine by the procedure used. It has, however, been found that administration of desoxycorticosterone acetate to patients causes an immediate and

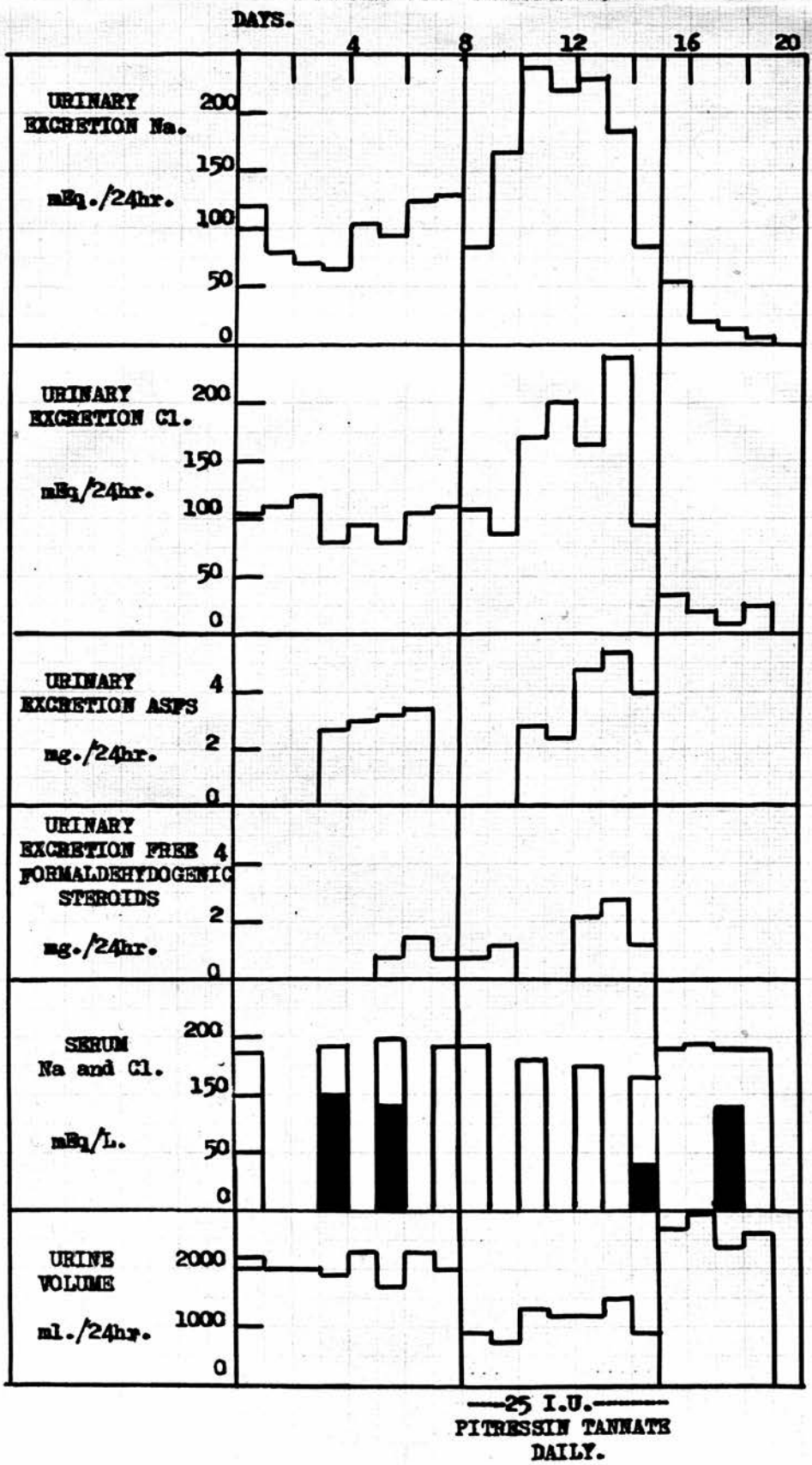
striking increase in the amounts of ASFS in the urine (Tompsett 1953) and the results presented in the methods section of this paper demonstrate that desoxycorticosterone glucoside produces the same effect. Thus it seems possible that estimation of ASFS in urine may afford a means of measuring some metabolic product of desoxycorticosterone which was not included in the urinary steroid fractions measured by Genest.

The fact that disturbances in sodium metabolism in the body may cause variations in the amounts of ASFS excreted in the urine has been confirmed in the course of investigations on the effects of daily injection of pitressin tannate on the serum sodium and chloride levels of experimental subjects.

Figure 20 shows the fall in the concentration of sodium and chloride in the urine of a twenty four year old male subject who received ten international units of pitressin tannate in oil daily for a period of seven days. The effects on salt and water metabolism are evident. A decrease in the blood levels of both sodium and chloride was accompanied by a striking excretion of these ions in the urine and a decrease in the twenty four hour urine volume. An increase in the amounts of ASFS excreted was also noted and, at the same time,

Figure 20

Effect of daily injection of pitressin tannate on urinary excretion of sodium, chloride, acid stable formaldehydogenic steroids and free formaldehydogenic steroids by a male subject and on urine volume and serum concentrations of sodium and chloride.



estimations of free formaldehydogenic steroids in the urine by the method of Lloyd and Lobotski (1950) gave higher results than were obtained during the preliminary control period.

7. CONCLUSIONS

1. In four hypertensive subjects and three normotensive subjects carefully studied during a control period and after removal of salt from the diet there was no consistent variation in the amounts of 17-ketosteroids present in the urine during the whole time of examination. Thus it appeared that a correlation did not exist between sodium chloride intake and 17-ketosteroid output.

2. Similarly no consistent change in the amounts of 17-ketogenic steroids in the urine of two hypertensive patients occurred after salt ingestion had stopped. It is therefore concluded that urinary excretion of 17-ketogenic steroids was not related to salt intake.

3. Measurement of acid stable formaldehydogenic steroids daily in the urine of six normal and three hypertensive subjects indicated that the urinary excretion of these compounds was influenced by the amount of salt in the diet and indicated that the adrenal cortex was concerned in the conservation of body salt supplies which was evident when sodium chloride was removed from the food.

GENERAL SUMMARY AND CONCLUSIONS

In Section 1 of this paper were reported the changes in electrolyte and nitrogen excretion which were noted when six hypertensive and six normotensive persons were subjected to abrupt and rigid restriction of dietary salt. It was observed that, during the period of salt deprivation, a temporary increase in the amount of potassium in the twenty four hour urine collections consistently occurred.

Consideration of the effects of administration of adrenal cortical hormones, as reported in the literature showed that the changes which were noted in the excretion of sodium, potassium, chloride and nitrogen might have resulted from the action of such compounds.

In view of these facts the excretion of certain steroid metabolites in the urine of the normotensive subjects and three of the hypertensive subjects was studied both before and after removal of salt from the food. The urinary excretion of acid stable formaldehydogenic steroids by three normal persons and by two hypertensive patients increased for a short period during the time when salt was lacking in the diet.

Thus some indication that the necessity to conserve body salt supplies resulted in altered adrenal activity was obtained from the fact that

increased potassium excretion consistently occurred during the period of salt deprivation combined with the fact that, in certain cases, increased excretion of a particular fraction of the steroid metabolites present in urine was also evident.

In the past few years several workers have demonstrated that urinary extracts may possess sodium retaining properties. This has been shown both for normal persons and for patients suffering from various diseases associated with retention of salt and water (Chart and Shipley 1953, Luetscher et al 1952, Singer and Venning 1953). The identity of the sodium retaining factor has not been definitely established but, in a recent paper, Cope and Garcia LLaurada (1954) reported the finding in urinary extracts with sodium retaining properties of a compound which appeared to be identical in all respects with the new adrenal steroid, aldosterone.

This finding emphasises the importance of the contribution made to the field of scientific research concerned with sodium metabolism in health and disease by Tait and his associates when they isolated aldosterone from adrenal extracts. Their work and that of their Swiss collaborators has shown clearly the need for biological assay of the sodium retaining factor in the urine in problems concerned with the relation between adrenal activity and salt metabolism since they have demonstrated that this

factor is, in all probability, distinct from the corticosteroids previously studied.

It is finally concluded, therefore, that although in the present study some evidence has been obtained which connects the adrenal cortex with the striking conservation of sodium and chloride observed in hypertensive and normotensive subjects on a salt poor diet, the final solution of this problem cannot be found using any of the presently existing methods of chemical assay to estimate urinary steroids. To prove conclusively that body salt supplies are protected under such conditions by the sodium retaining properties of an adrenal cortical hormone methods of chromatographic fractionation and biological assay must be utilised to estimate the particular factor concerned.

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